Genetic and environmental predictors of psychiatric disorders and related traits

Power, Robert

Awarding institution:
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Robert Power

MRC Social, Genetic & Developmental Psychiatry Centre

Institute of Psychiatry

King's College London

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In Egypt's sandy silence all alone

Still searching how best to atone

Sweet Bubastis so loyal

Only for you did I toil

On a thesis of stone that time will foil

Contents

Abstract

Psychiatric disorders pose a major economic and health burden worldwide. Family and twin studies indicate strong genetic influences, with estimates suggesting substantial variance in liability is heritable. However known genetic risk variants for psychiatric disorders explain only a small fraction of the heritability estimated from twin studies, leaving the underlying genetic aetiology largely unknown. Given the wide range in prevalence, age at onsets, and gender ratios seen across psychiatric disorders it is reasonable to expect that different genetic architectures exist for each disorder. Thus the underlying genetic architecture of psychiatric disorders remains an open question, with large potential implications for identifying predictive genetic risk variants, redefining diagnostic criteria, and developing novel drug targets.

This thesis focuses on combining epidemiological, genetic and environmental data to answer the questions surrounding the genetic architectures of psychiatric disorders. Initial analysis of epidemiological measures surprisingly identified that major depression was not under negative selection, a finding that was then confirmed through analysis of genotypic data. These results suggested the potential role of gene-environment interactions as an adaptive mechanism for variants contributing risk to depression, and were followed up by studies focusing on identifying such interactions. The conclusion of the thesis was though no gene-environmental interaction could be found to explain how the risk variants for major depression avoided negative selection, this was likely due in part to substantial gene-environment correlation in the reporting and experiencing of environmental risk factors in psychiatric disorders which would confound such analyses. Further these gene-environment correlations likely confound epidemiological associations identifying 'environmental' risks, such as between cannabis and schizophrenia.

Statement of authorship

All work in this thesis was performed and written by Robert Power, with the following exceptions:

1) The published papers were both circulated amongst co-authors and underwent peer review prior to publication, leading to editing of manuscript and suggestions for additional analyses.
2) The datasets used throughout the published papers were collected by others prior to analysis and underwent some quality control and cleaning (true for both phenotypic, epidemiological and genetic data).

1. Introduction

1.1.1 Outline

Psychiatric disorders pose a major economic and public health burden in developing and developed countries alike [Vos and others 2012]. Disorders such as schizophrenia, bipolar disorder, anorexia, autism and major depression are highly prevalent, with early mean ages of onset that lead to high levels of disability and morbidity, reduced reproductive fitness, and mortality. Family and twin studies indicate strong genetic influences, with estimates suggesting substantial variance in liability is heritable [e.g. Lee and others 2013b; Lichtenstein and others 2009]. This combination has long been noted as a paradox since natural selection should deplete genetic variation associated with reduced reproductive fitness [Keller and Miller 2006; Uher 2009]. This is further complicated as known genetic risk variants for psychiatric disorders explain only a small fraction of the heritability estimated from twin studies, leaving the underlying genetic aetiology largely unknown [Sullivan and others 2012]. Thus the underlying genetic architecture of psychiatric disorders remains an open question in the field, with large potential implications for identifying predictive genetic risk variants, redefining diagnostic criteria, and developing novel drug targets. This thesis will begin by outlining the nine major neuro-psychiatric disorders, then describing the relevant epidemiological and molecular genetic findings for each. The introduction will finish with summary of the publications which will compose the thesis.

1.1.2 Summary of neuropsychiatric disorders

1.1.2.1 Schizophrenia

Schizophrenia is a psychotic disorder that affects around 1% of the population [McGrath and others 2008], with a slightly higher prevalence in males than females. It is characterised by three main clusters of symptoms: positive symptoms such as hallucinations and paranoia; negative symptoms of anhedonia and catatonia; and disorganised thought processes with language and cognitive impairment [APA 1994]. Onset occurs most frequently in adolescents or early adulthood, on

average slightly earlier for males [Faraone and others 1994]. It has been associated with urbanicity, migrant status, latitude, and season of birth [McGrath and others 2008]. Cannabis use has also been associated with a twofold increased risk of schizophrenia [Arseneault and others 2004]. Schizophrenia is associated with increased mortality [Joukamaa and others 2001], in part due the suicide related symptoms of the disorder.

1.1.2.2 Bipolar disorder

Bipolar disorder is a mood disorder, similar to schizophrenia in that it often includes psychosis and affects around 1% of the population [Bebbington and Ramana 1995]. However the positive and negative symptoms of bipolar disorder manifest in a more cyclical manner, and it lacks the same level of cognitive impairment. Individuals switch between episodes of manic behaviour to episodes of depression [APA 1994]. Two subtypes are often defined: bipolar disorder type 1 where there are clear manic and depressive episodes; and bipolar disorder type 2 where less clear hypomania episodes exist among periods of depression. Bipolar disorder has been associated with risk factors such as divorce, financial problems and job loss [Hosang and others 2012], though this may be driven by prodromal symptoms as these are all common side effects of the reckless sexual and financial behaviour that can accompany manic episodes. Unlike schizophrenia it does not show a robust association with lower socioeconomic status, with some studies even finding the opposite direction of affect [reviewed by Bebbington and Ramana 1995]. Interestingly, some studies have suggested an association with increased cognitive abilities in individuals with bipolar disorder [Gale and others 2013; MacCabe and others 2010]. Despite this, increased mortality exists and suicide is prevalent among those affected [Osby and others 2001].

1.1.2.3 Major depressive disorder

Major depressive disorder (MDD), also known as unipolar depression, is the most highly prevalent psychiatric disorder (alongside the often co-morbid anxiety disorders) affecting ~15% of individuals over their lifetime usually at twice the prevalence in women as in men [Hasin and

others 2005; Kessler and others 2003]. With most individuals experiencing recurrent episodes throughout life [Mueller and others 1999], it is now the 2nd leading cause of disability worldwide [Vos and others 2012]. It is a highly heterogeneous disorder, defined by reduced mood and energy, inability to experience enjoyment, changes to diet and sleep patterns, feelings of guilt or worthlessness, and suicidal thoughts [APA 1994]. Along with excess mortality and increased risk of suicide [Angst and others 2002], it is associated with worse clinical outcomes when co-morbid with health problems such as cardiovascular disease and cancer [Barth and others 2004; Satin and others 2009]. MDD can occur at any age with many life events associated with onset such as puberty, menopause, childhood maltreatment, childbirth, and divorce [Angold and others 1998; Freeman and others 2004; Greenwald and others 1989; Hosang and others 2012; Thapar and others 2012a].

1.1.2.4 Autism

Autism is a lifelong developmental disorder that is manifests in early childhood, with a prevalence of ~0.1% and found around 4 times as frequently in males than females [Fombonne 2005]. Prevalence has increased over recent decades though this is believed to be driven largely by improved ascertainment and broader diagnostic categories [Rutter 2005]. Symptoms fall into three broad categories: social impairment; reduced ability to communicate; and repetitive behaviour [APA 1994]. Autism is known to exist on a spectrum of severity and affected individuals will not necessarily have symptoms from all three categories, though severe impairment in one will of course impair the others. It is also often co-morbid with intellectual disability.

1.1.2.5 Attention deficit hyperactivity disorder

Attention-deficit hyperactivity disorder (ADHD) usually manifests in childhood, and is defined by developmentally inappropriate levels of hyperactivity, impulsiveness and inattention [APA 1994]. These symptoms are generally considered to give rise to three subtypes of ADHD: predominantly inattentive type (high inattention, low hyperactivity), predominantly hyperactive-impulsive type

(low inattention, high hyperactivity), and combined type (high on both symptom dimensions). ADHD was initially believed to be a childhood disorder but is now recognised as frequently spanning from preschool age into adolescence and adulthood [Barkley 2008]. The prevalence of ADHD has been estimated at around ~5% in children and adolescents [Polanczyk and others 2007] and at around 2.5% in the adult population [Simon and others 2009]. It has been suggested that ADHD is associated with lower cognitive ability [Kuntsi and others 2004]. Associations with prenatal environment, such as maternal alcohol use or stress, have been reported but are not firmly established [Thapar and others 2012b].

1.1.2.6 Eating disorders

Anorexia nervosa and bulimia nervosa represent the two most prominent eating disorders. Anorexia nervosa is characterised by extreme dieting and avoidance of food while bulimia nervosa is characterised by binge eating followed by purging, with a combined prevalence of around 1% and anorexia nervosa being the rarer of the two [Hoek and van Hoeken 2003; Hudson and others 2007]. Both have a much higher prevalence in women, usually with onset in the late teens [Lucas and others 1991]. Prevalence in men may be higher than estimated though due to underreporting [Strother and others 2012], and it is known that prevalence of anorexia varies greatly across countries and cultures [Hoek 2006; Pavlova and others 2009]. Anorexia is a particularly serious disorder due to its extremely high mortality rate, the highest of any psychiatric disorder [Sullivan 1995; Zipfel and others 2000].

1.1.2.7 Substance abuse and addiction

Substance abuse covers a large range of disorders that each vary greatly in their prevalence, usually as a result of the availability of the substance both in terms of geography and generation. The most common substances are those legally available such as alcohol and tobacco, with lifetime prevalences estimated at 17% and 24% respectively [Breslau and others 2001; Hasin and others 2007], but extend to illicit drugs such as cannabis, opiates, or cocaine. Addiction is a complex trait

that can be conceived on many levels that may not apply equally to every substance, such as the willingness to try new or illegal experiences, choice of social group and peers, ability to tolerate the substance, susceptibility to addiction in terms of personality, and susceptibility to addiction based on biological disposition to the substance. One of the more consistent findings from studies of addition and substance abuse is that males are at greater risk [Becker and Hu 2008].

1.1.2.8 Alzheimer's disease

Alzheimer's disease is the most common form of dementia, accounting for up to 75% of cases [Lobo and others 2000]. It is characterised by rapid cognitive decline with an onset in late life, though early-onset forms exist. Symptoms include confusion, mood swings, language problems and memory loss. It is associated with a wide variety of risk factors [reviewed by Qiu and others 2009], including alcohol use, blood pressure, and poor social or mental activity throughout life. Ultimately cognitive functioning decreases until death.

1.1.3 Expectations

Clearly psychiatric disorders represent a wide variety of behavioural problems and impairments. They range greatly in prevalence from 0.1% to 15%, often appear much more frequently in women (e.g. anorexia and depression) or men (autism and substance abuse), and have onsets that range from just after birth to old age. Given the wide range in prevalence, age at onsets, and gender ratios seen across psychiatric disorders it is reasonable to expect that different genetic architectures exist for each disorder. In the next two sections I will evaluate the epidemiological and molecular evidence for the underlying genetics of these disorders, and build a consensus on where the causal variants exist.

1.2 Genetic epidemiology

Epidemiological studies can provide insight into the underlying genetics of any trait without requiring molecular genetic data. Twin and adoption studies can be used to test the relative impact of genes and environment on a trait, estimating the heritable component to variation between

individuals. Impact on fecundity gives us an insight into the selection on a trait, and so how likely it is that causal variants can survive in the population. Paternal aging is associated with increased burden of *de novo* mutations in the sperm, meaning an association with older fathers may reflect the importance of novel mutations. And lastly, non-random mating in the population with respect to a trait gives us insight into the likely distribution of causal variants. In this section I will expand on each of these epidemiological features and their bearing on psychiatric disorders.

1.2.1 Twin studies

The format of a twin study is intuitively appealing, as it naturally controls several variables. Twins are born at the same time in the same family, and hence should share a similar environment. They also share genes, 100% in identical or monozygotic twins (MZ) and 50% in terms of fraternal or dizygotic twins (DZ). These percentages refer of course to genetic differences relative to the general population (where one is 0% identical to an unrelated individual), as most of the human genome is unchanging across all individuals. This difference in genetic similarity between twins is due to MZ twins coming from a single fertilised egg splitting into two, while DZ come from two separately but simultaneously fertilised eggs. A comparison of concordance rates between and within DZ and MZ twin pairs gives an insight into the role of genetics in the disease. If a disease was entirely genetic in nature, it would be expected that MZ twins were 100% concordant, and DZ twins 50% discordant reflecting their genetic differences. Even when that's not the case, the level of genetic difference between MZ and DZ twins can be used to estimate genetic, shared and non-shared environmental factors through simple simultaneous equations when correlations of twins is known. This follows from the equations below.

$rMZ = h^2 + c^2$

$rDZ = h^2/2 + c^2$

$Hence\ 2(rMZ - rDZ) = h^2$

Here the rMZ reflects the correlation between MZ twins, which should be the heritability of the trait (h^2) plus any shared environmental influences (c^2). rDZ is the correlation between DZ twins which is the same as that of MZ, except reflecting only half the heritability as they share only half their genetic predisposition. As such, the correlations between MZ and DZ twins can be used to determine the heritability. The use of more advanced model fitting can then expand on these calculations, and provide confidence intervals for the estimates of genes and environment on the trait.

The majority of psychiatric traits have high heritabilities, though range from 0.37-0.81. The highest heritabilities are in schizophrenia, bipolar disorder, ADHD and autism. In reviews of 12 twin studies of schizophrenia and bipolar disorders the heritabilities were estimated to be 0.81 and 0.75 respectively [Smoller and Finn 2003; Sullivan and others 2003], though population studies give lower estimates at around 0.6 as well as reporting significant genetic overlap between them [Lichtenstein and others 2009; Wray and Gottesman 2012]. A meta-analysis of 20 studies of ADHD showed a heritability of 0.76 [Faraone and others 2005], though estimates have been found to be higher in children than in adults [Boomsma and others 2010; Larsson and others 2013]. A similar meta-analysis of 30 twin studies of autism estimated a heritability of 0.80 [Ronald and Hoekstra 2011], with some evidence for substantial overlap in the heritability for severe autism cases as those on a more moderate spectrum [Robinson and others 2011]. Studies of Alzheimer's disease and anorexia nervosa have suggested moderate heritabilities, of 0.58 and 0.56 respectively [Bulik and others 2007; Gatz and others 2006]. The heritability of substance abuse shows large ranges, perhaps due to the differing definitions of addiction across cultures and countries, or the availability of addictive substances. Alcohol addiction ranges from 0.48–0.66, nicotine from 33–71%, cannabis 51–59%, and cocaine from 42 to 79% [reviewed by Agrawal and Lynskey 2006]. The lowest heritability are found for major depression at around 0.35 in both twin and population

studies [Sullivan and others 2000; Wray and Gottesman 2012], though some studies suggest it is substantially higher in early-onset recurrent cases [Levinson 2006; McGuffin and others 1996].

Note that these estimates reflect additive genetic effects, and so ignore gene-environment or gene-gene interactions, dominant and recessive effects, and *de novo* mutations. They also rely on several assumptions, most importantly the equal environments assumption that MZ twins are not treated more similarly than DZ twins. Twin estimates also assume no assortative mating on the trait studies and that twins accurately represent this trait in the population, both of which may vary across disorders. Hence, twin estimates of heritability are not without some caveats when used as a benchmark for the role of genetics in any given disorder.

1.2.2 Reproductive fitness

Natural selection shapes the genetic architecture of any heritable trait. A genetic variant's ability to stay at a given frequency is dependent on its impact on an organism's reproductive fitness. The mutation-selection balance hypothesis suggests that deleterious genetic variants remain in the gene pool as new mutations are introduced at a pace equal to the selection against existing copies of the variant. An extension of this is the common disease common variant (CDCV) hypothesis where by a large number of risk variants with individually very small effects could escape selection. Antagonistic pleiotropy may also play a role, whereby genes that increase liability to a deleterious trait are beneficial under some circumstances, compensating for the negative selection in affected individuals. Selection shapes the genomic landscape of any trait, such as the number of risk alleles, their effect size, and their penetrance.

Calculating the impact of psychiatric disorders on reproductive fitness is usually performed presenting a fecundity ratio (FR), comparing the fecundity of the disorder to that of the general population. Studies have largely focused on schizophrenia, and their results were meta-analysed in a recent review [Bundy and others 2011]. Patients with schizophrenia were found to have

significantly reduced fitness (FR=0.39) while siblings showed a slight reduction (FR=0.96), with the effect being larger in males in both instances. They reported that parents of schizophrenic individuals showed no significant difference in FR, while one of the included studies reported that male offspring of schizophrenic individuals show reduced fecundity [Svensson and others 2007]. Outside of schizophrenia, bipolar disorder has not as consistently shown strong associations with reduced fecundity [MacCabe and others 2009; Mansour and others 2011; McGrath and others 1999], though there is some evidence for reduced fecundity in autism, eating disorders and addictions [Uher 2009].

Some studies have focused on potential benefits of psychiatric risk variants, specifically creativity. Several early attempts were made to quantify the overlap of creativity and mental health, looking for an increased levels of creative professions in those affected by and the relatives of those with psychiatric disorders [Andreasen 1987; Herbert 1959; Jamison 1989; Juda 1949; Ludwig 1992; Post 1994]. For the most part these studies reported such an association, though were restricted by biased ascertainment and small sample sizes. In a more recent family study of 300,000 individuals [Kyaga and others 2011], those with bipolar disorder and healthy siblings of people with schizophrenia or bipolar disorder were overrepresented in creative professions, but not for individuals with or related to those with major depression. In a subsequent larger study, Kyaga and others [2013] found that, except for bipolar disorder, individuals in creative professions in general were no more likely to be diagnosed with psychiatric disorders than controls, though being an author was specifically associated with risk of psychiatric illness. Creative professions were found to be more frequent among first-degree relatives of patients with schizophrenia, bipolar disorder, anorexia nervosa, and for siblings of those with autism. These findings have suggested a potentially shared heritability between creativity and psychiatric disorders, particularly bipolar disorder and schizophrenia, might exist.

1.2.3 Paternal age

Paternal age has been associated with increased *de novo* mutations in a father's offspring [Kong and others]. This is assumed to be due to the increased number of DNA replications that occur within sperm precursor cells compared to those that develop into eggs. This provides more opportunities for mutations to occur in male germ-line cells than those in females as they age. Thus an association with paternal age can provide insight into the role of *de novo* mutations in the genetic architecture of a trait. A further mechanism has been suggested, whereby those mutations that benefit sperm in their competition with each other to be passed on to the next generation are not necessarily those that benefit the offspring, and so may lead to deleterious traits [Goriely and others 2013]. This of course could maintain *de novo* mutations across generations.

Early studies showed clear associations between both autism and schizophrenia and paternal age, specifically males fathering offspring over ~40, suggesting *de novo* mutations play a role in these disorders [Brown and others 2002; Durkin and others 2008; Hultman and others 2011; Malaspina and others 2001; Reichenberg and others 2006; Zammit and others 2003]. Bipolar disorder showed a weaker association, though it was stronger in early onset cases [Frans and others 2008]. Follow up studies showed that paternal age acts as a risk factor across generations, with grandpaternal age associated with autism even when paternal age is matched for [Frans and others 2013]. Maternal grandfather's age also appears to contribute to risk in schizophrenia [Frans and others 2011], perhaps suggesting the role of mutations on the X chromosome. Two new studies have examined the impact of paternal age across a variety of disorders in Swedish and Danish populations [D'Onofrio and others 2014; McGrath and others 2014]. These showed paternal age effects across all disorders except eating disorders, including autism, ADHD, bipolar disorder, schizophrenia, suicide attempt, substance abuse, intellectual disability, and low educational attainment. Offspring of very young mothers and fathers were also found to be at increased risk, presumably due to environmental causes rather than genetic differences. It is also possible that older or younger

parents may differ genetically in some way that predisposes their offspring to mental illness. For example, males that take longer to find partners and have children may be further along the schizophrenia spectrum. Thus some have argued that it is actually the father's age at the birth of their first child rather than age at the birth of any individual child that is associated with risk [e.g. Pedersen and others 2014].

1.2.4 Non-random mating

Non-random mating can affect the genetic architecture of a trait in several ways. As mentioned above twin studies assume mating at random, as positive assortative mating (where individuals mate more often with similar individuals) can decrease estimates of heritability by increasing the genetic similarity of DZ twins above 0.5. Positive assortative mating also increases the number of homozygous individuals, pushing individuals to the extreme of the spectrum and potentially impacting the effects of selection. Positive assortative mating has long been shown with measures of personality [Zietsch and others 2011], though it remains unclear to what extent this results from cohabitation of partners. Increased cohabitation and assortative mating has been shown for severe psychiatric disorders, with very high levels reported for schizophrenia and bipolar disorder [Lichtenstein and others 2006; Thomsen and others 2013].

Positive assortative mating can lead to inbreeding defined as when individuals have a greater level of homozygosity in their genome than expected by chance, usually with reference to being identical by descent from a common ancestor. Inbreeding results in homozygosity across the genome, which can expose rare recessive variants that otherwise would not be expressed. Deleterious variants will be selected out of the population but, as the strength of negative and positive selection is greater on those of dominant effect, existing deleterious variants are more likely to be recessive. Hence inbreeding can expose deleterious mutations that otherwise would have been heterozygous. Inbreeding is associated with increased rates of illness, most commonly with reduced cognitive functioning [Jensen 1983; Morton 1978; Woodley 2009].

1.3 Molecular genetic findings

Genetic studies of psychiatric disorders have rapidly expanded over the previous decades, mainly due to the huge changes in technology and cost that have revolutionised the field of genetics as a whole. This ranges from the first linkage studies of the co-segregation of disease and genotype in families, to genome-wide association studies comparing cases and controls for differences at hundreds of thousands or even millions of markers across the genome, to the more recent advances in whole genome sequencing. In this section I will give a brief description of each of these methods and their current findings in psychiatry.

1.3.1 Linkage studies

The first insights into the genetics of psychiatric disorders came from linkage studies. These focus on genotyping genetic polymorphisms within family pedigrees with multiple affected individuals. These studies look for the co-segregation of disease and genotypes across generations of family members, isolating the region in which the causal variant exists. Within this framework the results of multiple pedigrees can be combined to increase power, though this relies on the same mutation having an effect across families. These approaches had huge success with those disorders showing the classic Mendelian inheritance patterns of recessive or dominant effects, of which many include psychiatric aspects such as intellectual disability and autism [Betancur 2011]. For more complex manifestations of psychiatric disorders the results for linkage analyses were less impressive. Large scale meta-analyses of linkage studies for schizophrenia and bipolar disorder showed only one significant finding on chromosomes 2p12-q22.1 predisposing to schizophrenia [Lewis and others 2003; Segurado and others 2003], later found again in a larger meta-analysis including some of the same studies [Ng and others 2009], and was unlikely to explain risk to a large proportion of cases. In contrast to this, Alzheimer's disease showed clear linkage with regions surrounding rare autosomal dominant variants of unusually large effect in the amyloid beta precursor protein (*APP*), presenilin 1 (*PSEN1*) and presenilin 2 (*PSEN2*) genes in families with early success in the severe

form of early-onset Alzheimer's disease [Bertram and Tanzi 2008]. Variants in the apolipoprotein E (*APOE*) were also identified that increased risk in late-onset Alzheimer's disease [Corder and others 1993], a gene that would reappear in genome-wide association studies along with several other common variants that contribute risk [Lambert and others 2013].

1.3.2 Genome-wide association studies (GWAS)

1.3.2.1 Methodology

With the somewhat disappointing results for complex disorders from linkage studies, alternate methods were explored that moved away from the family design best suited for Mendelian disorders. The common disease common variant (CDCV) hypothesis was put forward, broadly suggesting that many high frequency but low effect variants might contribute to risk to complex disorders, thus explaining how they avoided negative selection, manifested in complex inheritance patterns, and allowed for genetic and phenotypic heterogeneity within disorders. To attempt to identify some of these common variants, genome-wide association studies (GWAS) were implemented. These restricted to looking at previously identified common variants (frequency>1%) across the genome, usually in the form of bi-allelic single nucleotide polymorphisms (SNPs). In the case of diseases, DNA samples from case and control groups are analysed using specially developed genotyping chips that contain anywhere from hundreds of thousands to millions of SNPs from across the genome. Each SNP is then tested to see if one variant, or allele, is significantly more frequent among cases than controls. Due to the co-inheritance of segments of the genome over generations, correlations between SNPs allow one to 'tag' local variation in the region. This means that while an association might be found with a SNP, it may actually be tagging a truly causal variant in the region rather than having a direct biological impact on risk. As such only relatively few SNPs need to be genotyped in order to capture a large amount of the common genetic variation across the genome. Further, these correlations between SNPs, or

linkage disequilibrium, also allows for the imputation of additional SNPs not directly genotyped themselves but able to be filled in based on known correlations with those SNPs that have been.

GWAS provided a novel method for identifying risk variants across the genome, without relying on prior biological knowledge of gene function needed to select a gene for candidate studies. This also had the perhaps unexpected outcome of identifying numerous variants in 'gene deserts', without any clear functional impact on gene expression or structure suggesting at a much more complex system. The other benefit was that GWAS could move away from the pedigrees required for linkage analyses, removing some the constraints on recruitment and also alleviating concerns that the ascertainment of familial cases might give insight into a different aetiology compared to sporadic cases. The weakness of the GWAS method was the risk of false positives, the cause being twofold. First, the wide recruitment of unrelated cases and controls runs the risk of subtle population differences between the two groups e.g. an excess of cases from at risk minority groups. This can lead to the identification of SNPs that while highly significant are informative only of ancestry, not the underlying biology of the disease. To account for this many studies restrict recruitment to ethnically homogenous groups, e.g. where participants reported themselves as white with all grandparents were born in the UK. To account for more subtle influences of population stratification, principal component analysis is frequently used to construct covariates capturing correlations of SNPs across the genome. These have been shown to clearly capture subtle ancestry differences with high accuracy [Novembre and others 2008]. The second source of false positives is more intrinsic to the nature of GWAS, with the testing of hundreds of thousands of markers leading to a very large number of SNPs expected to be significant at $p<0.05$ by chance (since this reflects a 1 in 20 chance of the result occurring at random). To account for this a genome-wide significance level is often implemented at p=5E-8, estimated to account for the number of tests across the genome [Dudbridge and Gusnanto 2008]. This high threshold for significance means large sample

sizes are required to have adequate power to detect true effects, and a lower threshold is often used to identify SNPs at a suggestive significance to be taken forward for replication.

1.3.2.2 Findings

The initial GWAS in psychiatric disorders showed little to no success in identifying causal risk variants, unlike some somatic disorders [Wellcome Trust Case Control Consortium 2007].There were several exceptions to this, mostly in those psychiatric disorders where the biology was better understood. As mentioned the first GWASs into Alzheimer's disorder quickly identified novel findings [Harold and others 2009; Hollingworth and others 2011; Lambert and others 2009; Naj and others 2011] despite relatively small sample sizes, including regions originally identified in candidate studies. GWAS of nicotine addiction also identified variants in nicotine receptor genes [Tobacco and Genetics Consortium 2010; Liu and others 2010; Thorgeirsson and others 2010] and for alcohol usage and dependence in alcohol dehydrogenase genes previously associated with ability to break down alcohol [e.g. Bierut and others 2012; Gelernter and others 2014].

While the initial disappointing results for most disorders was taken by some as evidence against the common disease common variants hypotheis, efforts were made by researchers to pool samples together into meta-analyses (or mega-analyses, with data analysed together) in order to increase power to detect SNPs of small effect [e.g. Purcell and others 2009]. This approach came to fruition most clearly with the Psychiatric GWAS Consortium (later the Psychiatric Genomics Consortium or PGC), which in 2011 published two GWAS on schizophrenia and bipolar disorder [Ripke and others 2011; Sklar and others 2011]. The schizophrenia analysis combined 9,394 cases and 12,462 controls from 17 studies and identified 7 risk loci for schizophrenia, including the major histocompatability complex (MHC) region that had previously been suggested in earlier candidate gene studies [McGuffin 1979]. The analysis of bipolar disorder identified two genome-wide significant loci in a sample of 7,481 cases and 9,250 controls from 12 studies. Both studies were able to show that among the top associations, there was a highly significant excess of SNPs where

the direction of effect was concordant within the discovery and independent replication datasets suggesting that with greater sample sizes some of these SNPs might reach genome-wide significance. A future meta-analysis of the PGC schizophrenia studies with several thousand additional cases and controls from Swedish samples would prove this true [Ripke and others 2013a], identifying 13 more loci at genome-wide significance. A similar trend was seen in Alzheimer's disease, with the pooling of samples into the GERAD consortium of 17,008 cases and 37,154 controls to identify 20 associated loci [Lambert and others 2013], and as previously mentioned GWAS to some extent verified previously known variants from linkage studies. These results showed that once large enough sample sizes had been achieved, GWAS could identify novel associations and insights into the aetiology of psychiatric disorders.

Not all large GWAS collaborations yielded such successes though. The PGC analysis of MDD failed to identify any genome-wide significant associations, and the concordance between discovery and replication samples for the top SNPs was much weaker, despite a similar sample size of 9,240 cases and 9,519 controls [Ripke and others 2013b]. Similar negative findings were found for ADHD and autism [Neale and others 2010; Smoller and others 2013], though here the sample sizes were smaller. However the success in schizophrenia motivated the collection of genotyped samples for new disorders, both within and without the PGC, including anorexia nervosa [Boraska and others 2014; Wang and others 2011], cannabis use [Verweij and others 2013], Tourette's [Scharf and others 2013], OCD [Stewart and others 2013] , anti-social behaviour disorder [Tielbeek and others 2012], anxiety [Trzaskowski and others 2013], insomnia [Byrne and others 2013] and borderline personality [Lubke and others 2013]. Though most of these GWAS led to negative findings, a few successes stand out, namely post-traumatic stress disorder [Xie and others 2013] and in a very large meta-analysis of educational attainment [Rietveld and others 2013], as a proxy for cognitive functioning. Despite the current abundance of negative findings, increasingly large samples seem likely to lead to a greater and greater number of associations

1.3.3 Polygenic methods

For the most part even where genome-wide significant associations have been identified in GWAS, they still explain only a very small proportion of the variance in risk for psychiatric disorders. Hence several methods have been designed to make use of GWAS data to estimate the amount of variance explained when the effects of variants across the genome are combined. One of these was alluded to briefly in the above section, whereby concordance between the direction of effect in the discovery sample's top SNP associations is compared to the direction of effect in the replication sample (known as a sign test). However most of the methods discussed here focus not on the top SNPs from a GWAS but on all available SNPs. This is due to the stringent threshold for genome-wide significance and the presumption that many truely causal SNPs of small effect might not meet it, yet their cumulative effect could explain a large amount of the variance in risk.

1.3.3.1 Polygenic risk profile scoring

The first method to be discussed is polygenic risk profile scoring (PRS). This is similar to a sign test in that it requires a discovery sample and a replication (or target) sample. In the discovery sample a GWAS is performed, defining the risk allele and effect size for each SNP. In the target sample the number of risk alleles an individual carries is summed into a score, with each SNP weighted by its effect size. This is then used as a predictor of risk to determine how much of the variance between cases and controls is explained by the PRS. The PRS is often defined using various p-value thresholds for the inclusion of SNPs from the discovery GWAS, e.g. four scores using SNPs with $p<0.001$, $p<0.05$, $p<0.2$, and $p<0.5$. As more SNPs are included, the greater the likelihood that all SNPs of true effect will be captured. However including more SNPs also includes more with no true effect and so adds noise, causing a levelling off of the amount of variance explained. The first application of PRS was in the International Schizophrenia Consortium, which showed ~2% of the variance could be explained across samples [Purcell and others 2009]. They were also able to show that PRS for schizophrenia could also predict bipolar disorder vs. controls with a slightly reduced effect size. Future studies within the PGC would show that about 7% of the variance for

schizophrenia was predicted by PRS [Ripke and others 2011], 3% for bipolar disorder [Sklar and others 2011], and 0.6% for MDD [Ripke and others 2013b]. Cross disorder analysis also showed that schizophrenia, bipolar disorder and MDD PRS all predicted across each other, with some more weakly predicting into autism and ADHD [Smoller and others 2013]. More complex Bayesian approaches to PRS have also estimated the number of independent risk variants for schizophrenia to be between 6,300–10,200, accounting for 32% of the variance in risk [Ripke and others 2013a].

1.3.3.2 Genomic-relatedness-matrix residual maximum likelihood analysis

An alternate polygenic method, genomic-relatedness-matrix residual maximum likelihood analysis (GREML), was designed not for prediction of risk but to estimate what proportion of variance is captured by SNPs, thus giving a heritability estimate based on known genetic variants rather than from twin models [e.g. GCTA, developed by Yang and others 2011]. This method works by estimating the relatedness of all pairs of individuals in the dataset, and testing how much of the similarity in phenotype within pairs is accounted for by genetic similarity (relatedness). Relatedness here is defined as the proportion of the genome for which they have the same genotypes. It is important to note that the results of this analysis do not reflect the true heritability of a trait, as it only includes that heritability captured by the included SNPs while usually ignoring the role of rare or structural variation. It does however act as a benchmark for the maximum amount of risk detectable in an infinitely powered GWAS. An analysis within the PGC showed schizophrenia, bipolar disorder, MDD, ADHD, and autism all had relatively similar SNP-heritabilities at around ~20-25%, with the highest in ADHD (28%) and the lowest in autism (17%) [Lee and others 2013b]. GREML analysis can also be applied using bivariate models to estimate the co-heritability of two disorders captured by SNPs, with the key benefit of not requiring individuals to have data on both phenotypes [Lee and others 2012]. MDD, schizophrenia and bipolar disorder showed highly significant co-heritabilities at around 10-15%, as well as slightly lower co-heritabilities for MDD-ADHD and schizophrenia-autism [Lee and others 2013b]. Secondary

analyses suggested that while a large proportion of the heritability consistently came from genes annotated as related to brain function, it also unexpectedly showed that within disorders different studies did not overlap in their genetics entirely e.g. the heritability of MDD in study 1 might only show a co-heritability of 60% with the heritability of MDD in study 2, presumably due to heterogeneity across studies. Other studies have shown significant heritabilities for Alzheimer's (24%) [Lee and others 2013a] and Tourette's and OCD (58% and 37%, with significant genetic correlation between them) [Davis and others 2013]. Other studies have shown that SNP-heritability substantially overlaps across populations [de Candia and others 2013; Yang and others 2013]. These findings broadly are in agreement with those from PRS studies, suggesting that common risk variants contribute substantial risk to psychiatric disorders despite the individual risk variants not yet being identified. Presumably this is due to a large number of variants of small effect, with the results suggesting that these variants contribute non-specific risk to psychiatric disorders in general rather than uniquely to one disorder.

1.3.3.3 Runs of homozygosity

The last polygenic method discussed here is the analysis of runs of homozygous markers across the genome. An excess of homozygosity across the genome reflects inbreeding, whether through more recent events, such as consangous marriages, or among more distant ancestors. It has been shown that by looking for sections of consecutive homozygous markers more accurately captures inbreeding over using simply percentage of genome is homozygous [Keller and others 2011]. Homozygosity not only reflects inbreeding but also exposes recessive variants, those that have a non-additive effect with number of alleles an individual carries. A fully recessive variant is only expressed when an individual carries a copy on both chromosomes, though variants with partially recessive effects exist. As this can hide deleterious alleles from being expressed, natural selection acts to increase the frequency of dominant beneficial alleles and selects out more quickly dominant deleterious alleles. As a result, one expects deleterious traits to show an excess of recessive alleles.

Hence if these runs of homozygosity (ROH) are associated with a trait or disorder, it is evidence that the disorder has experienced historical selection against it. This informs both on its negative effect on ancestral human reproductive fitness, and that a recessive model of risk may be more appropriate when attempting to identify risk variants. Such an association has been suggested by several studies of psychiatric disorders, most prominently in schizophrenia [Keller and others 2012], intellectual disability and autism [Gamsiz and others 2013]. However, due to the very small effect sizes expected future studies require very large sample sizes in order to identify such an association [Keller and others 2011].

1.3.5 Whole Genome Sequencing

In the last few years GWAS have been followed up by whole-genome sequencing (WGS). This approach has become more popular as the cost has decreased and next generation technologies have been developed. While GWAS relies on using common genetic markers to tag local variation, WGS allows for the identification of the causal variant directly. It also allows for the analysis of rare variants, in particular *de novo* mutations that could never be found on a genotyping chip of known variants. The downside of this approach is that the sheer number of variants to test increases dramatically. This has several draw backs: by increasing the computational burden of analyses; by inflating the burden of multiple testing and so the threshold for significance; by including not just common variants but also rare variants where the power to detect significance is greatly reduced; and by not restricting to known variants and so increasing the risk of a incorporating sequencing errors. As such many methods for analysing WGS data have focused on analysing burden of rare variants across segments of the genome, or have returned to the format of linkage studies by leveraging pedigree information to investigate co-segregation of disease and variant. These methods often have led to a focus on variants within genes, where their impact on the protein can be estimated and weighted for. As such some studies have focused on exome sequencing, targeting only the portions of the genome that transcribe to mRNA. Within psychiatric disorders, Alzheimer's

again has led the way with the discovery of several variants in exome and genome sequencing studies [Guerreiro and others 2012; Pottier and others 2012]. And within other psychiatric disorders, the results are again less impressive to date. The most recent large scale analysis of 2,536 schizophrenia cases and 2,543 controls' exome sequences suggested cases showed a polygenic burden of disruptive mutations, particularly concentrated in previously associated gene pathway [Purcell and others 2014]. Several studies of exome sequencing in autism came out in 2012, implicating rare and *de novo* mutations though unable to identify individual loci definitively [Iossifov and others 2012; Neale and others 2012; O'Roak and others 2012; Sanders and others 2012]. These studies, as well as for schizophrenia [Fromer and others 2014], found an excess of *de novo* mutations amongst cases, particularly those with later paternal ages, providing evidence that these novel mutations may play a crucial role in psychiatric disorders.

1.3.6 Structural variation

As well as point mutations in the genome, where one base pair is replaced by another, there is the possibility of structural variation where base pairs or segments of the genome are deleted or duplicated. While many forms of structural changes exist, current genetic research has focused on larger copy number variants (CNV) that can be *de novo* or remain in the population as CNV polymorphisms. Schizophrenia has been seen as highly prevalent within the syndromic CNVs, such as 22q11 cardiofacial syndrome. As well as these syndromic effects, several large and rare CNVs of large effect are known to convey risk to schizophrenia, though also numerous other disorders such as autism, intellectual disability and epilepsy [reviewed inSullivan and others 2012]. Intellectual disability and autism both have a high burden of large structural variants or in a large number of cases [Folstein and Rosen-Sheidley 2001; Sanders and others 2011; Vorstman and others 2006] , sometimes established as the cause such as in the case of fragile X syndrome. As well as the identification of large CNVs, GWAS data can be used to identify smaller CNVs. This is done by looking at the signal intensity for the alleles, and looking for stretches of homozygosity or a 2:1

ratio of heterogeneity. These methods have been used to look at burden of CNVs across the genome for various psychiatric disorders. Such an association established in several studies of schizophrenia [Szatkiewicz and others 2014; Walsh and others 2008; Xu and others 2008], ADHD [Williams and others 2012; Williams and others 2010; Yang and others 2013] and autism [Pinto and others 2010], particularly among de novo CNVs and those in genic regions [Kirov and others 2012; Szatkiewicz and others 2014]. The results for bipolar disorder are more mixed [Grozeva and others 2010; Malhotra and others 2011; McQuillin and others 2011; Priebe and others 2012; Zhang and others 2009], while only one association for MDD with CNV burden has been reported [Rucker and others 2013]. Alzheimer's again shows concordance across different methods with associations with variants across the APP region [McNaughton and others 2012; Rovelet-Lecrux and others 2006].

1.4 Summary and outline of thesis

1.4.1 State of psychiatric genetics findings

These molecular and epidemiological findings have started to give insight into the genetic architecture of psychiatric genetics. While individual risk variants are still relative few for most disorders, a few patterns have started to emerge. Structural, rare and common variants for schizophrenia, bipolar disorder and autism appear to cluster in genes with brain function. Polygenic approaches have shown that common variants account for around ~15-30% of the variance in psychiatric disorders, approximately a third or half of the heritability. This suggests that as GWAS studies increase in size they will have sufficient sample size to identify many more common variants of small effect. Studies of cumulative burden of rare and *de novo* structural and point mutations across the genome also suggest that larger samples will yield associations with individual variants. Epidemiological evidence for paternal age effects suggests these approaches may have the greatest success for schizophrenia and autism. However progress may depend on the speed at which whole genome sequencing becomes viable, as well as the development of

methodologies for analysing very rare variants. Hopefully the disorder with the greatest success, Alzheimer's disease, will act as a template whereby rare, common and structural variants converge onto specific regions, presenting biological insight and drug targets.

This success will no doubt come through the ability to generate larger and larger sample sizes for a variety of disorders, something that is already occurring through cheaper genotyping/sequencing technologies and the acceptance of the need for international collaborations. In the meantime, several approaches can make use of existing data to gain a better picture of the underlying genetics of psychiatric disorders. My thesis will make use of six papers across three areas of research to gain novel insight into the genetic architecture of psychiatric disorders. The following sections will outline the contents and objectives of each.

1.4.2 Impact on reproductive fitness

In the first section of my thesis I will examine two ways in which psychiatric disorders can impact reproductive fitness and behaviour. The first paper, "Fecundity of patients with schizophrenia, autism, bipolar disorder, depression, anorexia nervosa, or substance abuse vs their unaffected siblings" [Power and others 2013b], focuses on a systematic analysis of the impact of psychiatric disorders on fecundity, as measured by number of children in both those diagnosed with a disorder and unaffected relatives. As discussed above this has huge impact on how causal variants are maintained in the population, and so the distribution of frequencies and effect sizes. The second paper "A recessive genetic model and runs of homozygosity in major depressive disorder" focuses on major depression following the results of the previous paper, looking for molecular support for a lack of negative selection on depression in the Psychiatric Genomics Consortium's sample of over 9000 each of MDD cases and controls with genome-wide association data [Power and others 2014a]. Together they paint a picture of where we might expect to identify causal variants in molecular genetic studies, and how these expectations differ across psychiatric disorders.

1.4.3 Gene-environment interactions

Following the results of the previous section showing potential evidence for beneficial effects of genetic risk variants for MDD, the next section will focus on gene-environment interactions and whether they could provide an answer to why these variants appear to sometimes increase an individual's fitness. It is clear that some individuals succumb to environmental risk factors while others do not. Whether risk variants for a disorder predispose individuals only in certain environments seems plausible, though the evidence for such gene-environment interactions is often highly contested.

In this section I first test the 'Mismatch hypothesis' which suggests environmental risks may interact with each other differently based on an individual's genetic background of 'plasticity' variants in a paper titled "The interaction between child maltreatment, adult stressful life events and the 5-*HTTLPR* in major depression" [Power and others 2013c]. In a second paper, I look not at testing for gene-environment interactions but to stratify cases and controls into equivalent levels of environmental stress exposure, in a paper "Genome-wide association analysis accounting for environmental factors through propensity-score matching: Application to stressful live events in major depressive disorder" [Power and others 2013a]. Both approaches aim to move beyond the conventional gene-environment interaction analysis, which has to date produced limited returns.

1.4.4 Untangling genetic from environmental risk

Following from the findings of the previous section, I explore how the relationship between environmental and genetic risk factors is complicated by the possibility not just of interactions but also correlations. There are several ways in which this can occur, with individuals selecting their own preferred environments and having their environments react to them. This can contribute to gene-environment correlation, particularly with respect to behavioural traits, and creates a problem for disentangling the causation of associations in the epidemiology of psychiatric trait. In two papers, "Estimating the heritability of reporting stressful life events captured by common

genetic variants" [Power and others 2013d] and "Genetic predisposition to schizophrenia associated with increased use of cannabis" [Power and others 2014b], I test for an underlying heritability for the known 'environmental' psychiatric risk factors of stressful life events and cannabis use, and look at how this heritability may overlap with the heritability of psychiatric disorders themselves.

1.4.5 Conclusion

Psychiatric genetics is on the verge of major advances, largely due to the massive increase in available data. As yet there are still many unanswered questions about the underlying aetiology of these disorders, such as their impact on reproductive fitness, the extent to which we can dissect heterogeneity within disorders through genetics, and the complex relationships between genes and environment. Overall this thesis will combine epidemiological, genetic and environmental data to attempt to answer some of these questions.

ORIGINAL ARTICLE

Fecundity of Patients With Schizophrenia, Autism, Bipolar Disorder, Depression, Anorexia Nervosa, or Substance Abuse vs Their Unaffected Siblings

Robert A. Power, BSc; Simon Kyaga, MD; Rudolf Uher, MD, PhD, MRCPsych; James H. MacCabe, PhD, MRCPsych; Niklas Långström, MD, PhD; Mikael Landen, MD, PhD; Peter McGuffin, FRCP, FRCPsych, PhD; Cathryn M. Lewis, PhD; Paul Lichtenstein, PhD; Anna C. Svensson, PhD

Context: It is unknown how genetic variants conferring liability to psychiatric disorders survive in the population despite strong negative selection. However, this is key to understanding their etiology and designing studies to identify risk variants.

Objectives: To examine the reproductive fitness of patients with schizophrenia and other psychiatric disorders vs their unaffected siblings and to evaluate the level of selection on causal genetic variants.

Design: We measured the fecundity of patients with schizophrenia, autism, bipolar disorder, depression, anorexia nervosa, or substance abuse and their unaffected siblings compared with the general population.

Setting: Population databases in Sweden, including the Multi-Generation Register and the Swedish Hospital Discharge Register.

Participants: In total, 2.3 million individuals among the 1950 to 1970 birth cohort in Sweden.

Main Outcome Measures: Fertility ratio (FR), reflecting the mean number of children compared with that of the general population, accounting for age, sex, family size, and affected status.

Results: Except for women with depression, affected patients had significantly fewer children (FR range for those with psychiatric disorder, 0.23-0.93; $P<10^{-10}$). This reduction was consistently greater among men than women, suggesting that male fitness was particularly sensitive. Although sisters of patients with schizophrenia and bipolar disorder had increased fecundity (FR range, 1.02-1.03; $P<.01$), this was too small on its own to counterbalance the reduced fitness of affected patients. Brothers of patients with schizophrenia and autism showed reduced fecundity (FR range, 0.94-0.97; $P<.001$). Siblings of patients with depression and substance abuse had significantly increased fecundity (FR range, 1.01-1.05; $P<10^{-10}$). In the case of depression, this more than compensated for the lower fecundity of affected individuals.

Conclusions: Our results suggest that strong selection exists against schizophrenia, autism, and anorexia nervosa and that these variants may be maintained by new mutations or an as-yet unknown mechanism. Bipolar disorder did not seem to be under strong negative selection. Vulnerability to depression, and perhaps substance abuse, may be preserved by balancing selection, suggesting the involvement of common genetic variants in ways that depend on other genes and on environment.

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Author Affiliations are listed at the end of this article.

PSYCHIATRIC DISORDERS HAVE long puzzled researchers by defying the expectations of natural selection.[1] From an evolutionary viewpoint, selection should remove genetic variants that reduce an individual's ability to reproduce ("fitness") because they will produce fewer offspring to inherit those variants. However, psychiatric disorders do not fit this model. They combine substantial heritability with moderate to high prevalence, early age at onset, and reduction in fitness compared with the general population. Several hypotheses have been put forward on how psychiatric disorders survive in the population,[2-5] but the mechanisms that maintain the genetic variants conferring susceptibility to these disorders remain unclear.

Insights into the form of selection pressure on psychiatric disorders may help direct research toward identifying specific causal pathways. The large disparity between heritability estimates from family studies and the amount of variance in psychiatric disorders explained by identified risk alleles has become known as the "missing heritability."[6] Current genome-wide association studies are based on the

common disorder–common variants hypothesis, which presumes that many low-risk high-frequency alleles directly lead to highly prevalent complex disorders. Evidence suggesting an alternative reason why risk alleles remain in the population could explain why more causal variants have not been discovered through linkage or association studies.

Several alternatives to the common disorder–common variants hypothesis exist. Autism, bipolar disorder, and schizophrenia have been associated with increased paternal age.[7-10] Older paternal age carries a risk of an increased number of de novo mutations during spermatogenesis,[11] which in turn may lead to deleterious phenotypes in the next generation.[12] Alternatively, deleterious genes that exist in the population may only recently have come under purifying selection, as changes in selection pressures have made them detrimental in the present environment. A competing hypothesis is that causal genetic variants may not be entirely deleterious but may also confer benefits (eg, creativity[13]) and that balancing selection maintains these variants in the population at optimal frequency. Possible mechanisms for balancing selection include heterozygote advantage, pleiotropic antagonism, and gene-environment interactions. A specific form of balancing selection is sexual antagonism, where a trait may be beneficial to one sex but harmful to the other.

To our knowledge, this analysis is the first to evaluate the fecundity of affected individuals and their siblings for multiple psychiatric disorders, including schizophrenia, autism, bipolar disorder, depression, anorexia nervosa, and substance abuse. Previous research has focused solely on psychosis, and a recent meta-analysis[14] found no overall increase in the fecundity among siblings of those with schizophrenia. Similar evidence is missing for most other psychiatric disorders. The aim of this study was to examine sibling fecundity to evaluate evidence for the aforementioned theories for the continued prevalence of psychiatric disorders (**Table 1**). Under balancing selection, we expected that unaffected siblings of those with psychiatric disorders would enjoy increased reproductive fitness because they would benefit from the positive effects of the same genetic variants that contributed to psychiatric disorders in their kin. Sex-specific effects on sibling fecundity may provide evidence for sexual antagonism, with the same genetic variants benefiting one sex at the cost of the other. For de novo or recent highly penetrant mutations, no reduction in the fecundity would be expected in unaffected siblings (as long as affected siblings are diagnosed). If these disorders were affected by multiple variants that were ancestrally neutral but deleterious in today's environment, we expected to see decreased fecundity in siblings due to an increased probability of sharing those variants and the unsuited environment.

Table 1. Hypotheses of Maintenance of Genes for Psychiatric Disorders and Predicted Results for Fecundity in Affected Individuals and Their Unaffected Siblings

	Predicted Result for Fecundity	
Hypothesis	Affected Individuals	Unaffected Siblings
Balancing selection	Decreased	Increased in proportion to the decrease in affected individuals
Sex-dependent selection	Decreased to a greater extent in one sex	Decreased in the same sex as that of affected individuals but increased in the opposite sex
De novo mutation	Greatly decreased	No change
Ancestral neutrality	Decreased	Decreased to a lesser extent owing to shared genes and environment

METHODS

POPULATION DATABASES

The data set was drawn from the Multi-Generation Register, which includes children born in Sweden since 1932 and their biological parents.[15] All individuals alive in 1960 and all births from this point onward were recorded in the register. Paternity is assumed to be the husband of the mother at the time of birth or "by acknowledgment" for unwed mothers. This data set was linked to the Swedish Hospital Discharge Register, which covers virtually all psychiatric hospitalizations since 1973 in Sweden[16] and has been previously validated.[17,18] It also includes partial coverage of outpatient diagnoses from 2001 onward. The use of this database has been approved by the ethics committee at the Karolinska Institutet, Stockholm, Sweden. Diagnoses were established according to the *International Classification of Diseases* (*ICD*), *Eighth Revision* (1973-1987), *Ninth Revision* (1987-1996), and *Tenth Revision* (1996 onward). These registers were linked using an individual's unique national registration number. The analyses were restricted to 2 356 598 individuals born in Sweden between 1950 and 1970, for whom most of their adult life was covered in the Swedish Hospital Discharge Register and who would have completed most of their reproductive life span (age 40 years for the youngest individuals). Only individuals for whom both parents were known were included in the birth cohort, although this limitation was not applicable when calculating the number of offspring.

DISORDERS CLASSIFICATION

Six disorders were examined, including schizophrenia, autism, bipolar disorder, depression, anorexia nervosa, and substance abuse (including alcohol use disorder). The 6 disorders were chosen to differ in terms of prevalence, severity, heritability, and their distribution between men and women. In all cases, previous studies[19-24] using the Multi-Generation Register and the Swedish Hospital Discharge Register were used as a basis for the selection of *ICD* codes. Following the validation of bipolar disorder in the Swedish Hospital Discharge Register by Sellgren et al,[24] 2 or more diagnoses over a lifetime were required o be included. For all other disorders, only a single lifetime diagnosis in the Swedish Hospital Discharge Register was required. Svensson et al[23] had previously examined schizophrenia in the Multi-Generation Register and the Swedish Hospital Discharge Register in an earlier (nonoverlapping) birth cohort. Schizophrenia was defined by 1 or more recorded diagnoses of code 295 in *ICD-8* and *ICD-9* and by codes F20, F23.1, F23.2, and F25 in *ICD-10*. Autism was defined by code 299.0 in *ICD-9* and by codes F84.0, F84.1, F84.5, and F84.9 in *ICD-10* and was not defined in *ICD-8*. Bipolar disorder was defined by code 296 (excluding code 296.2) in *ICD-8*, by code 296 (excluding code 296.1) in *ICD-9*, and by codes F30 and F31 in

Table 2. Epidemiological Details for 6 Psychiatric Disorders Among the Cohort of 2 356 598 Individuals Born in Sweden Between 1950 and 1970

Psychiatric Disorder	No. of Affected Individuals	Prevalence, %	Female-Male Ratio	No. of Children Among the Cohort, Mean No. of Affected	No. of Unaffected Siblings
Schizophrenia	18 890	0.80	1:1.5	0.61	28 644
Autism	2947	0.12	1:2	0.56	4471
Bipolar disorder	14 439	0.61	1.5:1	1.48	22 986
Depression	81 295	3.44	1.4:1	1.78	119 645
Anorexia nervosa	3275	0.14	10.4:1	1.46	5172
Substance abuse	55 933	2.37	1:2.3	1.49	81 592

ICD-10. One of these was allowed to be code 296.2 in *ICD-8* or code 296.1 in *ICD-9*. Depression was defined by codes 296.0, 296.2, and 298.0 in *ICD-8*; by codes 296.2, 296.3, and 298.0 in *ICD-9*; and by codes F32 and F33 in *ICD-10*. Anorexia nervosa was defined by code 306.5 in *ICD-8*, by code 307.1 in *ICD-9*, and by code F50.0 in *ICD-10*. Last, substance abuse was defined by codes 303 and 304 in *ICD-8*; by codes 303, 304, 305.1, and 305.9 in *ICD-9*; and by codes F10 through F19 (excluding subsection 0.5, diagnosing substance abuse with psychosis) in *ICD-10*. Diagnoses were made on discharge by the treating physician. No hierarchical diagnostic practice was used; hence, individuals with comorbidity could appear in more than 1 category. A large amount of comorbidity existed within the sample, with affected individuals being diagnosed as having at least 1 other disorder in 45.7% of individuals with schizophrenia, 48.9% with autism, 71.7% with bipolar disorder, 26.4% with depression, 26.8% with anorexia nervosa, and 30.0% with substance abuse. High levels of comorbidity between psychiatric disorders is a common feature of cohort studies and has been described at length elsewhere.[25] For the objectives of this study, we first analyzed each disorder separately without accounting for comorbidities. A secondary analysis was then performed that corrected for comorbidities by analyzing all disorders simultaneously. Family identification codes were used to identify unaffected full siblings, while affected siblings were considered affected individuals, not siblings. The results for our identification of affected individuals and their siblings are summarized in **Table 2**.

MEASURE OF FECUNDITY

Data were analyzed using generalized estimating equations,[26,27] accounting for similarity in the number of children within families. To measure the reproductive fitness of each group, a fertility ratio (FR) was calculated based on the number of children individuals in that group had compared with the general population, correcting for the year of birth. For example, if the disease group had an FR of 0.5, it meant they had on average half as many children as the general population, while an FR of 2 meant they had twice as many. To permit testing for sex-specific effects and to avoid confounding by age differences at parenthood and the mean number of children, we compared affected men with the general population of men, and the same for affected women. Similarly, siblings of affected individuals were compared with only those in the general population of the same sex, with the additional requirement that they had at least 1 sibling, to account for any bias resulting from being a sibling. Socioeconomic effects have been shown to influence the risk for psychiatric disorders[28] and could potentially confound our analysis. To account for socioeconomic status, we corrected for both paternal and maternal education levels derived from the 1970 census data.[29] Parental education level rather than the self-education level was used to avoid reverse causation. Data on at least 1 parent were available for more than 95% of individuals.

To interpret the FRs of affected individuals and their siblings together, we followed the method by Haukka et al[30] and compared the prevalence of affected individuals and siblings plus that of their combined children. Therefore, we combined the estimated number of children from affected individuals and their siblings, and we then divided that sum by the estimated total number of children for the entire 1950 to 1970 birth cohort. The total estimated number of children was derived from the FR of each group (affected, sibling, and remaining population), multiplied by the mean number of children, and weighted for by each group's frequency in the birth cohort. Using the FR rather than the actual number of children of each individual, we corrected for the year of birth and the unequal distribution of affected individuals born each year. For this analysis, we used the FRs that were not corrected for comorbidities. All the analyses were performed using commercially available software (STATA, release 12; StataCorp LP).[31]

RESULTS

The mean (SD) number of children for the birth cohort was 1.76 (1.27). Paternal and maternal education levels were found to be significantly associated with fecundity. Because only minor differences were observed between the adjusted and nonadjusted estimates, only the adjusted estimates are presented herein. The mean (SD) number of siblings for the birth cohort was 1.68 (1.38).

SCHIZOPHRENIA

Individuals with schizophrenia had fewer children compared with the general population, with FRs of 0.23 (95% CI, 0.23-0.24; $P < 10^{-10}$) for men and 0.47 (95% CI, 0.46-0.48; $P < 10^{-10}$) for women (**Figure 1**). Sisters of affected individuals had a significantly increased number of children (FR, 1.02; 95% CI, 1.01-1.03; $P = .01$), while brothers of affected individuals showed significantly decreased fecundity (FR, 0.97; 95% CI, 0.96-0.99; $P < .001$) (**Figure 2**). When comorbidities were included in the analysis, the increased fecundity in sisters disappeared.

AUTISM

Individuals with autism had significantly fewer children, in men (FR, 0.25; 95% CI, 0.23-27; $P < 10^{-10}$) and women (FR, 0.48; 95% CI, 0.44-0.51; $P < 10^{-10}$). Brothers of affected individuals also had fewer children (FR, 0.94; 95%

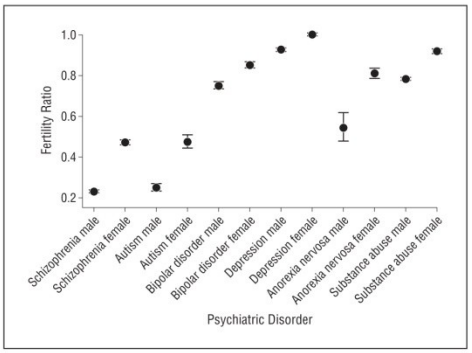

Figure 1. Fertility ratios for individuals with schizophrenia, autism, bipolar disorder, depression, anorexia nervosa, and substance abuse. A fertility ratio of 1 (highlighted) represents that of the general population.

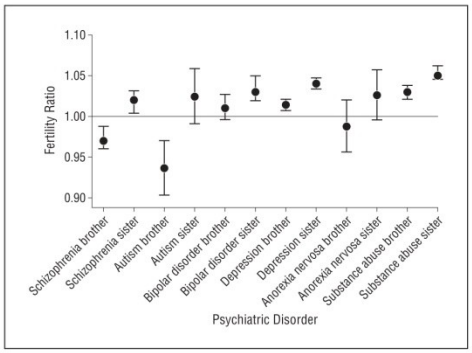

Figure 2. Fertility ratios for unaffected siblings of individuals with schizophrenia, autism, bipolar disorder, depression, anorexia nervosa, and substance abuse. A fertility ratio of 1 (highlighted) represents that of the general population.

CI, 0.90-0.97; $P < .001$), while sisters of affected individuals showed no significant difference from the general population. These results did not differ significantly when comorbidities were included in the analysis.

BIPOLAR DISORDER

Men and women with bipolar disorder had fewer children than the general population (male FR, 0.75; 95% CI, 0.73-0.77; $P < 10^{-10}$; female FR, 0.85; 95% CI, 0.84-0.87; $P < 10^{-10}$). Brothers of affected individuals showed no significant difference from the general population, while sisters of affected individuals had an increased number of children (FR, 1.03; 95% CI, 1.02-1.05; $P < 10^{-5}$). When correcting for comorbidity, the increased fecundity in sisters disappeared, and the reduced fecundity in affected individuals increased to just below that of the general population (male FR, 0.94; 95% CI, 0.92-0.96; $P < .001$; female FR, 0.95; 95% CI, 0.93-0.97; $P < .001$).

DEPRESSION

Men with depression had fewer children (FR, 0.93; 95% CI, 0.92-94; $P < 10^{-10}$), but women with depression showed no significant difference from the general population. Siblings of affected individuals had more children compared with the general population (brothers' FR, 1.01; 95% CI, 1.01-1.02; $P < .0001$; sisters' FR, 1.04; 95% CI, 1.03-1.05; $P < 10^{-10}$). This increased fecundity in siblings remained when comorbidities were accounted for, although the reduced number of children among affected men disappeared and the fecundity among women with depression increased (FR, 1.03; 95% CI, 1.03-1.04; $P < .001$).

ANOREXIA NERVOSA

Individuals with anorexia nervosa showed a reduced number of children in men (FR, 0.54; 95% CI, 0.48-62; $P < 10^{-10}$) and in women (FR, 0.81; 95% CI, 0.79-84; $P < 10^{-10}$). Neither the fecundity of brothers nor sisters differed from that of the general population. These results did not change after correction for comorbidities.

SUBSTANCE ABUSE

Men having a diagnosis of substance abuse had significantly fewer children than the general population (FR, 0.78; 95% CI, 0.78-79; $P < 10^{-10}$), as did women having a diagnosis of substance abuse (FR, 0.92; 95% CI, 0.91-0.93; $P < 10^{-10}$). Siblings of individuals with substance abuse had more children than the general population, with FRs of 1.03 (95% CI, 1.02-1.04; $P < .0001$) for brothers and 1.05 (95% CI, 1.05-1.06; $P < 10^{-10}$) for sisters. These values did not differ significantly when comorbidities were included.

COMMENT

To our knowledge, this is the first time the fitness of relatives has been examined for individuals with these psychiatric disorders (other than schizophrenia and bipolar disorder). Across disorders, affected men had a consistently greater reduction in fecundity than affected women. A similar sex difference was observed among siblings: sisters of individuals with psychiatric disorders had more children than their brothers. The degree of fecundity reduction in affected individuals and the associated increase in the fecundity among siblings differed by disorder, which suggests that different types of psychiatric disorders are under different selection pressures.

STUDY LIMITATIONS

Our conclusions rely on the assumption that the fecundity measured truly reflects these individuals' reproductive fitness. Ideally, the number of grandchildren rather than children would be used to measure an individual's long-term fitness, and this should become possible in the future with the continuation of the Multi-Generation Register in Sweden. The fecundity in affected individuals may be decreased by effects of medication or hospitalization, which may have distorted our findings. Voluntary and compulsory sterilizations were historically performed in Sweden until the 1970s, including targeting those with

Table 3. Fitness Calculations of the Observed Proportions of Affected Individuals in the 1950 to 1970 Birth Cohort and the Estimated Proportions of Their Affected Children in the Next Generation

Psychiatric Disorder	Proportion of Affected Individuals		
	Observed in the 1950-1970 Birth Cohort	Estimated in the Next Generation	Change, %
Schizophrenia	0.022	0.016	−25
Autism	0.003	0.002	−28
Bipolar disorder	0.012	0.011	−6
Depression	0.092	0.093	1
Anorexia nervosa	0.003	0.002	−10
Substance abuse	0.066	0.064	−3

psychiatric disorders. It should be noted that most of those sterilized were women, while our results consistently show a greater reduction in male fecundity.[32,33] Last, children of those with a psychiatric disorder may go unrecorded as a result of stigma or chaotic lifestyles (eg, emigration), leading to an artificial lowering of their fecundity. However, these concerns are not applicable to unaffected siblings. Therefore, we believe that the number of children born to siblings of individuals with psychiatric disorders accurately reflects their fitness.

Is reproductive success today representative of reproductive success in the past? Recent reductions in child mortality and in the mean number of children per adult, as well as the increased use of contraceptives, raise questions about how well evolutionary fitness can be measured in the modern world. This is important because a trait's prevalence is dependent on the selection it has encountered in previous generations, not the current one. However, several reasons indicate why we would expect fitness today to reflect fitness in previous generations to some extent. First, changes in fecundity may reflect a biological or physiological difference in fertility that affects fitness regardless of culture or setting (eg, reduced sperm count). Second, it has been argued that the inability to attract a mate is responsible for low fitness in those with psychiatric disorders (reviewed by Keller and Miller[2]), supported by evidence showing that low marriage rates mediate the effect of psychiatric disorders on fitness.[34] If this is the case, then modern improvements in contraceptives and child mortality occur after the selection against individuals with psychiatric disorders has already occurred and so have less influence. Third, the extent of the impairment experienced by those with psychiatric disorders in traditional communities is high, suggesting that the effects are not culture specific.[35,36] Although our measure was suboptimal, we maintain that the large differences in fecundity suggest as close a measure of fitness as any other available in human data sets.

Finally, the generalization of a single etiology to a disorder may be unfounded. As described in the "Methods" section, considerable comorbidity exists between disorders. Longitudinal, family, and molecular studies[25,37,38] have demonstrated that psychiatric disorders show considerable overlap and share genetic risk variants. We attempted to tackle this by analyzing performing analyses with vs without accounting for comorbidities, and this had little effect (except for bipolar disorder). More problematic is the evidence for considerable genetic and phenotypic heterogeneity within psychiatric disorders.[39] Heterogeneity within disorders means that caution must be applied to generalizations of their genetic etiology.

SEX-SPECIFIC EFFECTS

Across disorders, the fecundity of affected men was lowered more than that of affected women, a finding that had been noted in previous studies.[14,40] This sex-specific effect suggests that psychiatric morbidity impairs interest or ability to find suitable mating partners or inhibits biological fertility to a greater extent in men. Several hypotheses attempt to explain why. Evolutionary theory suggests that male species have the potential for greater variance in reproductive success than female species.[41] This is assumed to result from their minimal investment being cheap compared with the minimal investment of women (ie, the cost of sperm production compared with 9 months of pregnancy). Because of this underlying difference in investment, it benefits women to be more selective in their choices of a mate. This can lead to mating systems often seen in mammals where "dominant" males have multiple mates, while others have none. Because of the greater variability in male fitness and the pressure on females to be selective, a genetic or environmental burden can have an exaggerated effect on a male's ability to find a mate. However, it should be noted that in this data set men had a lower variance in the number of children they had compared with women. Men also had fewer children on average than women, suggesting differences in reporting. A mother must be present at the birth of a child, but paternal uncertainty can exist even when a father is named. Another hypothesis is that fathers with a psychiatric disorder may be less likely to be recorded as the child's parent. Last, male fertility may be more susceptible than female fertility to sexual adverse effects of psychiatric treatment.

SCHIZOPHRENIA

Our findings for schizophrenia suggest a decrease in the fecundity of affected individuals similar to that found in a recent meta-analysis.[14] Our results suggest a strong selection pressure to remove genetic variants associated with schizophrenia from the population (**Table 3**). This is further evidence for the role of recent or de novo mutations in the genetic susceptibility to schizophrenia that have neither reached the frequency of nor existed long enough to be removed from the population.[12,42] This could act in conjunction with common variants' having too small an effect size to experience negative selection.[38] Our analysis also found a slight increase in the fecundity among sisters of affected individuals and a decrease in the fecundity among their brothers, although the increase in the fecundity of sisters disappears after correction for comorbidities. This trend in sibling fecundity is in agreement with several previous studies.[23,30,43,44] We have 2 potential hypotheses on why this might be the case. First, owing to the high heritability of schizophrenia and its

greater prevalence and severity among men, some brothers may have mild symptoms of schizophrenia but lack this diagnosis in our database. Second, schizophrenia may be the result of sexually antagonistic genes that are beneficial to female fitness at the expense of male fitness. This would be in agreement with the observations that schizophrenia affects men more severely than women and that the effect on fitness of shared genes in siblings is dependent on sibling sex. However, this may only be a partial explanation because the benefit to sisters does not compensate for the reduction in fitness seen in the affected individuals or brothers (Table 3).

AUTISM

Individuals with autism showed the greatest reduction in fecundity among all examined disorders. This was not unexpected because previous investigations have shown that few individuals with autism ever married or had children (eg, as demonstrated in the study by Larsen and Mouridsen[45]). The pattern of fecundity in affected individuals and siblings is similar to that of schizophrenia, with a slight increase in sisters' fecundity (nonsignificant herein) and a decrease in brothers' fecundity. As discussed for schizophrenia, this may be reflective of sexually antagonistic genes or undiagnosed symptoms in brothers. Therefore, we propose that rare highly deleterious variants and sexually antagonistic polymorphisms may contribute to the genetic disposition to autism. The similarity to schizophrenia is notable because it has been proposed that the autistic and psychotic spectrums reflect 2 extremes of social cognition.[46] It is unclear whether our results reflect a similar etiology or, as suggested by Crespi and Badcock,[46] they are opposite extremes of the same trait and come under the same stabilizing selection pressure. That both disorders show evidence for sexual antagonism supports the proposal by Crespi and Badcock that they are the result of sexual conflict.

BIPOLAR DISORDER

Unlike the other highly heritable disorders, bipolar disorder showed a low reduction in fecundity among affected individuals. This was accompanied by increased fecundity among sisters of affected individuals. However, owing to the large amount of comorbidity (see the "Methods" section), when this was corrected for, the fecundity of affected individuals increased to just below that of the general population. This agrees with the results of another study[34] of affective psychosis in Sweden but disagrees with other studies.[40,47,48] These studies have differed slightly in terms of diagnostic criteria, which may explain these discrepancies. It has been suggested that the introduction of lithium as a treatment for bipolar disorder has led to improved functioning and, as a result, greater fecundity in those populations where treatment is available.[49]

DEPRESSION

Notably, depression was an exception to the 5 other studied disorders. Female depressed individuals showed no difference in fecundity compared with the general population and had a slight increase in the fecundity after correction for comorbid disorders. Male depressed individuals showed a small decrease in the fecundity, although this too disappeared after correction for comorbidities. This contradicts the estimates of reduced fertility, especially in women, obtained from clinical samples of depressed individuals[50] and in a study[40] similar in design to ours using population registers in Denmark. The *ICD* classification used herein did not include identification of postnatal depression, and it is unclear to what extent this may lead to an increase in female fertility among individuals with depression. Furthermore, siblings of both sexes showed increased fecundity, and when this was taken into account, we found no selection acting against depression (Table 3). Rather, genes associated with depression seem to be maintained in the population by balancing selection because the cost to affected individuals is roughly equal to the benefit to their siblings. If this is the case, it would be the first strong evidence for balancing selection in a psychiatric disorder. The exact mechanism by which siblings benefit is beyond the scope of our analysis and is a line of future investigation. It has been proposed by Allen and Badcock[51] that depression may be adaptive in eliciting support from others. In parallel to the unique lack of negative influence on fecundity at the population level, depression stands out as a psychiatric disorder for which direct genetic associations have been most difficult to identify.[52-55] We propose that genetic studies in depression may benefit from the exploration of genetic and environmental dependencies that may contribute to balancing selection. An alternative explanation could be that environmental factors shared by siblings are associated with both an increased risk of depression and a higher fecundity.

ANOREXIA NERVOSA

Our analysis of anorexia showed a decrease in the fecundity among affected individuals but no difference in sibling fecundity. Our estimates of FRs in anorexia (0.54 in men and 0.81 in women) were less severe than a previous estimate from a clinical sample.[56] Our calculations suggest that anorexia is under weaker negative selection relative to schizophrenia and autism (Table 3).

SUBSTANCE ABUSE

Substance abuse was associated with reduced fecundity in affected individuals, but we found evidence for significantly increased fecundity in siblings of both sexes. Our findings suggest that this increased fecundity in siblings almost entirely accounts for the cost to affected individuals, with only a slight decrease (−3%) in the frequency of these individuals' genes predicted each generation. Considering that most drugs are a new environmental exposure when seen from an evolutionary perspective, it is possible that there has been insufficient time for selection to act on risk alleles. However, some evidence in the case of alcohol metabolism indicates that selection has affected different human populations differently.[57] Because alcohol abuse is the most frequent form of substance abuse in Sweden, we can as-

sume there has been sufficient time for some selection to have occurred.[58] It has also been suggested that substance abuse is associated with risk-taking behavior in both sexes, including sexual risk taking.[59]

SUMMARY AND RELEVANCE FOR PSYCHIATRIC GENETICS

The results of our analyses have several implications for future genetic studies. It seems likely that different evolutionary mechanisms underlie the persistence of the various psychiatric disorders. This in turn suggests that their genetic architecture may differ, so it is not surprising that the search for causal variants has proved more fruitful in some disorders than in others. More specifically, it seems that genetic variants conferring liability to schizophrenia, autism, and anorexia nervosa are under strong selection to be removed from the population. The continued high prevalence of schizophrenia and autism despite this strong negative selection, in combination with the aforementioned association with increased parental age, suggests that a high rate of de novo mutations may be maintaining these disorders in the population. The possibility of sexually antagonistic genes in schizophrenia and autism suggests that studies might benefit from male-only analysis, without women, who may be unaffected by risk alleles. Bipolar disorder did not seem to be under such strong negative selection and, after correcting for comorbidities, did not show sex-specific effects or changes in sibling fecundity.

Depression and, to a lesser extent, substance abuse, seems to be maintained by genes that are beneficial under some circumstances (ie, in siblings) but detrimental in others (ie, affected individuals). This suggests that gene-environment or gene-gene interactions have a large role in these disorders, for which some supporting evidence exists in depression.[60,61] This would decrease the power of studies comparing cases and controls, where many controls might also carry the genes that are "causal" for depression but not have the necessary genetic or environmental background risk factors to develop the disorder. Genes that interact with the environment may provide not only susceptibility to negative environments but also the ability to thrive in positive environments.[60] If the beneficial aspect of these genes is opposite to the disorder itself, rather than acting on a separate phenotype, then selecting high-functioning individuals as supercontrols might even increase the frequency of causal genetic variants in the controls. However, at this stage we have no evidence why siblings of individuals with depression or substance abuse would have increased fitness, and the observation could result from shared environmental factors uncorrected for in this analysis. Overall, a focus on case-only and exposed-only studies (eg, as in the study by Caspi et al[62]) might be more successful in disentangling the genetics of these disorders.

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Author Affiliations: Social Genetic and Developmental Psychiatry Centre, Institute of Psychiatry (Mr Power and Dr Uher), and Department of Psychosis Studies (Drs MacCabe, McGuffin, and Lewis), King's College London, London, England; and Departments of Medical Epidemiology and Biostatistics (Drs Kyaga and Lichtenstein) and Public Health Sciences (Dr Svensson), Centre for Violence Prevention (Dr Långström), and Division of Public Health Epidemiology (Dr Svensson), Karolinska Institutet, Stockholm, and Institute of Neuroscience and Physiology, The Sahlgrenska Academy at Gothenburg University, Gothenburg (Dr Landen), Sweden.
Correspondence: Robert A. Power, BSc, Social Genetic and Developmental Centre, Institute of Psychiatry, King's College London, De Creispigny Park, London SE5 8AF, England (robert.r.power@kcl.ac.uk).
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RESEARCH ARTICLE

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A Recessive Genetic Model and Runs of Homozygosity in Major Depressive Disorder

Robert A. Power,[1]* Matthew C. Keller,[2,3] Stephan Ripke,[4,5] Abdel Abdellaoui,[6] Naomi R. Wray,[7] Patrick F. Sullivan,[8,9,10,11] MDD PGC Working Group, and Gerome Breen[1,12,13]

[1]MRC Social Genetic & Developmental Psychiatry Centre, Institute of Psychiatry, King's College London, London, United Kingdom

[2]Department of Psychology and Neuroscience, University of Colorado at Boulder, Boulder, Colorado

[3]Institute for Behavioral Genetics, University of Colorado at Boulder, Boulder, Colorado

[4]Stanley Center for Psychiatric Research, Broad Institute of MIT and Harvard, Cambridge, Massachusetts

[5]Analytical and Translational Genetics Unit, Massachusetts General Hospital, Boston, Massachusetts

[6]Department of Biological Psychology, VU University Amsterdam, Amsterdam, The Netherlands

[7]The University of Queensland, Queensland Brain Institute, Brisbane, Australia

[8]Department of Medical Epidemiology and Biostatistics, Karolinska Institute, Stockholm, Sweden

[9]Department of Genetics, The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina

[10]Department of Psychiatry, The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina

[11]Department of Epidemiology, The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina

[12]NIHR Biomedical Research Centre for Mental Health, South London and Maudsley NHS Foundation Trust, London, United Kingdom

[13]Institute of Psychiatry, King's College London, London, United Kingdom

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*Correspondence to:
Robert A. Power, MRC Social, Genetic and Developmental Psychiatry Centre, Institute of Psychiatry, King's College London, London SE5 8AF, United Kingdom.
E-mail: robert.r.power@kcl.ac.uk

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Genome-wide association studies (GWASs) of major depressive disorder (MDD) have yet to identify variants that surpass the threshold for genome-wide significance. A recent study reported that runs of homozygosity (ROH) are associated with schizophrenia, reflecting a novel genetic risk factor resulting from increased parental relatedness and recessive genetic effects. Here, we explore the possibility of such a recessive model in MDD. In a sample of 9,238 cases and 9,521 controls reported in a recent mega-analysis of 9 GWAS we perform an analysis of ROH and common variants under a recessive model. Since evidence for association with ROH could reflect a recessive mode of action at loci, we also conducted a genome-wide association analyses under a recessive model. The genome-wide association analysis using a recessive model found no significant associations. Our analysis of ROH suggested that there was significant heterogeneity of effect across studies in effect ($P = 0.001$), and it was associated with genotyping platform and country of origin. The results of the ROH analysis show that differences across studies can lead to conflicting systematic genome-wide differences between cases and controls that are unaccounted for by traditional covariates. They highlight the sensitivity of the ROH method to spurious associations, and the need to carefully control for potential confounds in such analyses. We found no strong evidence for a recessive model underlying MDD.

Key words: runs of homozygosity; recessive risk model; major depression; inbreeding

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INTRODUCTION

Major depressive disorder (MDD) is one of the leading burdens of disease in the world, with a lifetime prevalence of ~15% [Kessler et al., 2003; Hasin et al., 2005]. It has been found to be moderately heritable, from 31% to 42% [Sullivan et al., 2000], though with greater heritability in severe, recurrent forms of the disorder [McGuffin et al., 1996; Levinson, 2006]. A recent mega-analysis of nine genome-wide association studies found no significant associations with individual genetic variants (Psychiatric Genomics Consortium MDD Working Group, 2012), compared to ~5 genome-wide significant associations in similar sized studies of other psychiatric disorders [Ripke et al., 2011; Sklar et al., 2011]. These association studies are conducted under an additive model, while the true effects of some risk variants may be recessive, for which individuals with two copies of an allele are at greater risk than would be predicted from twice a single allele's effect. In a fully recessive model only those with two copies of the risk allele are at risk, though there is also the possibility of partial recessive effects. As selection acts to remove deleterious alleles with respect to overall fitness from the population, genetic risk variants that are recessive can escape selection longer. Inbreeding within families (e.g. consanguineous marriages) often exposes such recessive alleles due to an increased likelihood of alleles at each locus being identical by descent. Until recently studies of inbreeding were focused on families or communities in which inbreeding is expressed relative to the founder generation, which is assumed to be unrelated and where inbreeding information was determined from self-reports or knowledge of pedigrees (pedigree inbreeding). For example, Rudan et al. [2003] found a higher incidence of six complex genetic diseases/disorders including MDD among Croatian villages with higher levels of pedigree inbreeding [Rudan et al., 2003]. By using genome-wide genotype data it is also possible to estimate an individual's inbreeding from more distant common ancestors to provide evidence for whether a recessive genetic model is more appropriate for a disorder.

One method to analyse the effect of inbreeding from genome-wide genotype data is to identify segments of continuous homozygous SNPs, reflecting blocks of the genome that are identical by descent from a common ancestor. Runs of homozygosity (ROH) capture inbreeding effects that are due to common or rare causal variants better than a simple measure of excess number of homozygous SNPs across the genome, which tends to only capture the recessive effects of common variants [Keller et al., 2011]. An association between percentage of genome covered by ROH (F_{ROH}) and schizophrenia has been reported [Keller et al., 2012]. Due to the possibility of genetic overlap between MDD and schizophrenia [Schulze et al., 2012; Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013], a similar association between F_{ROH} and MDD might be expected. However, MDD has a lower heritability ($h^2 \sim 0.37$, Sullivan et al., 2000) than schizophrenia ($h^2 \sim 0.81$, Sullivan et al., 2003), which should attenuate genetic relationships. Moreover, some authors have suggested that MDD may not be under negative selection and the casual genetic variants may be beneficial in some circumstances [Nesse, 1999; Watson and Andrews, 2002; Belsky and Pluess, 2009; Power et al., 2013]. Here we look at the association between MDD and SNPs in 9,238 cases and 9,521 controls across nine studies (Table 1) [Psychiatric Genomics Consortium MDD Working Group, 2012] under a recessive genetic model. We also use genome-wide estimates of inbreeding to look for a consistent difference between cases and controls across nine studies of MDD, in order to support a recessive model of the disorder.

MATERIAL AND METHODS

Sample

In this report, we analyzed individual data from the nine discovery samples [Ising et al., 2009; Sullivan et al., 2009; Lewis et al., 2010; Muglia et al., 2010; Rietschel et al., 2010; Shi et al., 2011; Shyn et al., 2011; Wray et al., 2012] of the PGC-MDD that together comprise 9,238 cases and 9,521 controls. Full sample details are given in the supplementary materials of the original analysis (Psychiatric Genomics Consortium MDD Working Group, 2012), and are outlined in Table 1. All subjects were of European

TABLE 1. Summary Statistics of Each of the Nine Studies and Results of Runs of Homozygosity Analysis

Study	N cases	N controls	Platform	Recurrence (%)	Ascertainment	Country	Number of SNPs[a]	Mean F_{ROH} (%)[b]	SD of Mean F_{ROH} (%)[b]	Traditional F estimate (%)[c]	Mean size of ROH (kb)	SD for Mean size of kb	Ref
RADIANT UK	1,625	1,588	Illumina 610k	97.1	Clinical	UK	263,728	0.10	0.41	0.1	3,294	1,373	Lewis et al. [2010]
MDD2000 OIMR-610k	433	751	Illumina 610k	57.6	Population	Australia	275,259	0.09	0.19	−0.01	3,376	1,049	Wray et al. [2012]
MDD2000 OIMR-317k	1,017	960	Illumina 317k[d]	51.4	Population	Australia	210,075	0.09	0.24	0.06	3,330	937	Wray et al. [2012]
Radiant + Bonn/Mannheim	935	1,290	Illumina 610k	74.2	Clinical	Germany	260,561	0.10	0.30	0.06	3,580	1,565	Rietschel et al. [2010]
GAIN	1,694	1,767	Perlegen 600k	50.7	Clinical/population	The Netherlands	199,749	0.12	0.36	0.15	3,780	1,840	Sullivan et al. [2009]
GenRED	1,030	1,253	Affymetrix 6.0	100	Volunteers	USA	253,826	0.08	0.18	0.09	3,238	763	Shi et al. [2011]
GSK	887	864	Illumina 550k	100	Clinical	Germany	281,029	0.16	0.67	0.14	3,732	1,711	Muglia et al. [2010]
MPIP	376	537	Illumina 317k	36.7	Clinical	Germany	215,393	0.11	0.30	0.04	3,533	1,424	Ising et al. [2009]
STAR*D	1,241	511	Affymetrix 5.0	74.3	Clinical	USA	155,428	0.10	0.40	0.17	3,362	783	Shyn et al. [2011]

[a]Genotyping was on either Illumina 317k or Illumina 370k SNPs, but only SNPs from Illumina 317k were used for imputation.
[b]After QC and pruning on linkage disequilibrium.
[c]F_{ROH} is the percentage of the genome covered by runs of homozygosity.
[d]This measure of *F* reflects an individual's observed number of homozygous loci compared to that expected under Hardy–Weinberg equilibrium.

ancestry (as determined from genome-wide genotypes). Cases were required to have diagnoses of DSM-IV lifetime MDD established using structured diagnostic instruments from direct interviews by trained interviewers (two studies required recurrent MDD and one recurrent, early-onset MDD) or clinician-administered DSM-IV checklists. Studies ascertained cases mostly from clinical sources, and controls were largely randomly selected from the population and screened for lifetime history of MDD.

Method of ROH Calling and Analysis

Genotyping was described in the supplementary materials in the original analysis (Psychiatric Genomics Consortium MDD Working Group, 2012). All samples were genotyped with single nucleotide polymorphism (SNP) arrays of greater than 200 K genome-wide SNPs, with analysis restricted to polymorphic SNP probes. In the original analysis, imputation to the CEU HapMap3 reference sample [Altshuler et al., 2010], 1,235,109 autosomal SNPs, was performed using Beagle 3.0.4 [Browning and Browning, 2009]. In order to perform an association analysis under a recessive model or call runs of homozygosity (ROH), imputed SNP dosage data was converted to discrete genotype calls, keeping those SNPs with a probability of at least 0.95. The use of imputed SNPs helped to increase similarity of genomic coverage across studies. SNPs with a missingness of >2% or minor allele frequency (MAF) <5% were removed, as were then individuals with missingness over 2%. Prior to analysis SNPs were pruned for LD within PLINK, removing any SNPs with an R^2 0.90 with any other SNP in a 50 SNP window. The use of imputed data in ROH has previously been shown to give similar results to those restricting to only genotyped SNPs [Keller et al., 2012]. The calling of ROH and percentage of genome covered by ROH per individual (F_{ROH}) were derived within PLINK [Purcell et al., 2007] following the same method found to optimally detect effects of autozygosity, as described in Howrigan et al. [2011]. In particular, we used a series of sliding windows across the genome to call ROH within each individual separately. The size of the windows was set to 65 consecutive SNPs, so any single SNP would be found in 65 different windows. If at least four (>5%) of these windows contained entirely homozygous SNPs, then the SNP in question could be included within a ROH. Within windows, one missing SNP was allowed. To avoid false positives, only ROH with a minimum of 65 consecutive SNPs covering 2.3 Mb were used when calculating F_{ROH}. In addition, the required minimum density in a ROH was set at 200 kb per SNP and the maximum gap between two consecutive homozygous SNPs was set at 500 kb. The estimate of the total genome captured was 2.77×10^9 bp. The analysis was performed by study, using F_{ROH} as a predictor of case-control status in a logistic regression. Percentage of SNPs missing, a SNP-by-SNP measure of homozygosity determined by PLINK's—het command, and the first five ancestry-informative principal components were used as covariates. The SNP-by-SNP measure of homozygosity was included to correct for differences in genomic-homozygosity levels unrelated to inbreeding, such as DNA quality or population ancestry. A mixed model was also examined combining all samples, using study as a random effect. This analysis was performed in STATA [StataCorp., 2011].

Genome-Wide Recessive Model

The genome-wide recessive model analysis used the autosomal dosage data converted to genotype calls as described above in the analysis of ROH. Analyses was performed in PLINK [Purcell et al., 2007], using the–recessive command. The first five ancestry-informative principal components were included as covariates. Analysis was restricted to autosomes. Each study was analysed separately and then a meta-analysis was performed for each SNP across studies (using fixed effect *P*-value in PLINK). As the risk allele is set as the minor allele by default, and this may differ by study for alleles at frequencies near 0.5, we used the minor allele in the analysis of imputed data from the whole sample as a reference. A *P*-value $<5 \times 10^{-8}$ was considered as genome-wide significant. For this significance cut-off, we had 90% power to detect a relative risk of 1.47 for the rare recessive genotype for SNPs with a MAF from 0.3 to 0.5. However, power decreased rapidly for those alleles with lower MAF, with 90% power to detect those with a relative risk of 1.81 and MAF of 0.2, or with a relative risk of 2.21 and MAF of 0.15. For those SNPs with lower MAF, power reduced rapidly for a recessive model. Calculations were performed using CaTS Power Calculator [Skol et al., 2006].

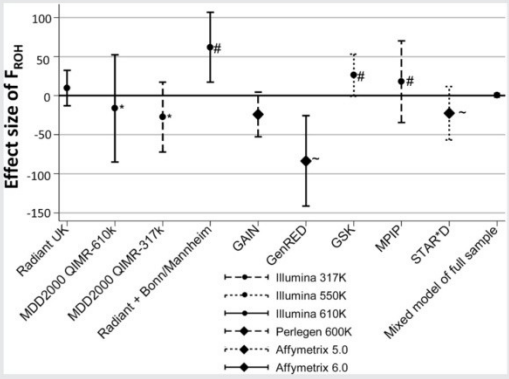

FIG. 1. Beta coefficient from logistic regression of F_{ROH} predicting MDD per study (with 95% CI, accounting for covariates). Positive effects suggest that ROHs are a risk factor for MDD. Note that though no combined effect in the mixed model of the full sample, in Illumina studies increased F_{ROH} was a risk factor ($P = 0.02$) and in non-Illumina studies it was protective ($P = 0.007$). Note also the consistency in studies from the same country: *Australian studies; ~American studies; #German studies.

RESULTS

Across all samples the average percentage of the genome covered by ROH (F_{ROH}) was 0.11% (95% CI 0.102–0.112; Table 1), similar albeit slightly lower than average F_{ROH} (0.15%) reported in an earlier report using the same parameters [Keller et al., 2012]. In our mixed model analysis across all samples with study as a random effect, we found no significant effect of F_{ROH} on MDD status. However, there was substantial heterogeneity in direction of effect across studies ($P = 0.001$, Fig. 1). Overall, four studies showed increased F_{ROH} in cases (one significantly, $P = 0.007$), while five studies showed increased F_{ROH} in controls (one where $P = 0.005$). Including further principal components (up to 20) and increasing ROH size (up to 170 SNPs) made no difference to the heterogeneity of the results.

To explore this apparent heterogeneity we examined two features of the included studies that might provide insight into the results. The first issue was potential poor matching of cases and controls, which we tested within the combined RADIANT German and Bonn-Mannheim sample. Here cases were recruited from both of these two studies, whereas the controls were collected and genotyped only as part of the Bonn–Mannheim study. However, excluding the RADIANT cases and restricting to only the matched Bonn–Mannheim study's cases and controls still resulted in a significant association with ROH ($P = 0.03$), as both sets of cases were found to have similar mean F_{ROH}. This tentatively suggested that the heterogeneity apparent across studies was not replicated within studies recruited from the same geographic region. Secondly, we were interested in the effects of genomic coverage on ROH. Cases and controls from the Queensland Institute of Medical Research (QIMR) were recruited as one sample but included as two independent cohorts, based on their genotyping platform (Illumina 317k and 610k chips). When we restricted the analysis to only those SNPs directly genotyped on both platforms (202,062 SNPs), we found that the mean F_{ROH} for the QIMR-610k sample reduced from 0.086% to 0.077%, compared to 0.078% for the QIMR-317k sample. This implies, as expected, that genotyping platform and genome coverage were influencing estimates of mean F_{ROH}.

To better understand potential sources of heterogeneity in our findings, we used a linear meta-regression with a study's effect size as the outcome and features of the studies individually analysed as predictors. We tested percentage of cases with recurrent MDD, mean F_{ROH} and genome-wide homozygosity, country of recruitment, presence of copy number variant probes on the platform, and genotyping platform as potential predictors of direction of effect (see Supplementary Table 1). Genotyping platform was nominally associated with effect size ($P = 0.05$), and the direction of effect of F_{ROH} differed when studies on non-Illumina platforms were analysed separately. Within the three non-Illumina genotyped studies, increased inbreeding was protective against MDD ($P = 0.007$) while within the six Illumina genotyped studies inbreeding was a significant risk factor for MDD ($P = 0.02$). We noted that when more than one study was recruited from a country, the direction of effect for ROH was consistent across all studies recruited from the same country (see Fig. 1). However, this was only a significant predictor in the meta-regression when distinguishing between German and non-German studies ($P = 0.02$). This level of confounding from genotyping and country likely reduced our power to detect a true effect of ROH and MDD.

In the meta-analysis of a recessive model of association, 929, 138 SNPs were analysed, though not all appeared in all nine studies due to differences in genotyping coverage. The most significant recessive association was for rs13261582 on chromosome 8 in an intergenic region between *SNTB1* and *HAS2* (odds ratio of 2.0 for the minor A allele, MAF 0.22, $P = 2.58 \times 10^{-6}$). However this SNP was only present in two studies (GenRED and STAR*D), and so is only supported by a subset of the sample. It did not appear among the reported top SNPs from the primary analysis of this dataset under an additive genetic model in the full sample ($P = 0.12$). The results of the meta-analysis also showed a lower median *P*-value than expected by chance (λ_{GC} 0.97, see Supplementary Figs. 1 and 2 for Manhattan and QQ plots). The λ_{GC} was 1.03 for SNPs with MAF > 0.2 and 0.90 for SNPs with MAF < 0.2, implying smaller differences of genotype frequencies between cases and controls than expected by chance for SNPS with low MAF. This is possibly the result of the less accurate imputation of rare alleles, or a lack of power.

DISCUSSION

Our analyses show systematic differences in F_{ROH} between cases and controls that differ in direction across studies. There are several explanations for these results, mostly highlighting limitations of this analysis. Firstly, we found systematic differences in mean F_{ROH} between studies. This is not unexpected and likely reflects the density of the genomic coverage and the accuracy of imputation, since SNPs were restricted to those with high quality imputation. A similar level of variation in F_{ROH} was observed in the Psychiatric GWAS Consortium's analysis of F_{ROH} across 17 studies of schizophrenia [Keller et al., 2012], though they did not report any heterogeneity of effect as a function of genotyping platform or country of recruitment. It seems unlikely that the heterogeneity of effect in the present study could be the result of differing SNP inclusion on the platforms, because such an explanation would imply systematic differences between cases and controls in the probability of homozygosity across SNPs as a function of platform. More likely in our opinion is the possibility that factors related to ascertainment of cases and controls differed across studies and influenced overall homozygosity. Such factors could include changes in homozygosity levels due to length and quality of DNA storage, or differences in ascertainment of cases and controls across populations. It is noteworthy that the two out of nine studies that genotyped controls independently of cases (GenRED and STAR*D) both showed higher F_{ROH} in controls than cases. Further, studies appear to cluster by country of origin and direction of the effect of F_{ROH}. All three German studies had increased F_{ROH} in cases for example, while the two Australian and two US studies all showed increased F_{ROH} in controls. This may reflect confounding demographic factors specific to each country. These unknown confounders, such as urban/rural status or religion, that influence both distant inbreeding (F_{ROH}) and MDD could explain the differences in effects between studies. A recent analysis of ROH and MDD in a partially overlapping sample of the GAIN study analysed here found exactly that. Religion confounded of the association due to reduced levels of depression but increased inbreeding in within the religious population of the Netherlands [Abdellaoui et al., 2013]. Certainly the initial hypothesis of this study, that an association with inbreeding would reflect negative selection on MDD and an excess of recessive causal mutations, seems an implausible explanation for the observed heterogeneity as the evolutionary cost of MDD status seems unlikely to have differed greatly among the ancestors of those included in the present study. Any of these explanations for the results of the F_{ROH} analysis may give some insight into why the original mega-analysis of these nine studies did not lead to any replicable genome-wide significant findings.

Our results from the genome-wide association analysis of MDD also produced no evidence for a recessive model, failing to produce any genome-wide significant associations. It is possible that our underlying model of recessive effect is unsuitable for an outbred population. Here we looked at a recessive effect for the minor allele, but two alternate models may also have been viable: compound heterozygosity and overdominance. Compound heterozygosity is an additional risk in individuals carrying two recessive but non-identical alleles within a genetic locus, while overdominance is the increased risk of homozygosity of any allele compared to being heterozygous. However, our analysis of both would have been restricted by low power and the use of biallelic markers, and were, therefore, not performed. Both the GWAS and ROH analyses suggest though that there is no underlying recessive model of MDD, at least not of large effect. Such an association was previously reported for schizophrenia in a similarly sized sample [Keller et al., 2012], showing an increase of risk for schizophrenia by 17% for every additional percentage of the genome covered by ROH and was taken as evidence for historical selection against schizophrenia risk variants. The lack of a similar association here adds molecular evidence to that from epidemiological studies suggesting MDD has little impact on reproductive fitness compared to other psychiatric disorders, and so is under substantially less negative selection [e.g., Power et al., 2013].

These results highlight that the analysis of F_{ROH} appears to be sensitive to systematic differences between studies that are ostensibly unrelated to MDD status, potentially give rise to either false positive or false negative results. This suggests there are genome-wide differences in homozygosity and/or inbreeding between populations that are not corrected for by methods such as ancestry-informative principal components. We recommend the use of large combined samples in the analysis of F_{ROH} as a predictor of traits and disorders, due to the high risk of spurious associations within one study. Preferably such analyses should be done with access to data on potential social and demographic confounders. One possible further improvement might be the development of novel methods for analysing ROH, particularly in imputed genotype data where probability for homozygosity across a region is available. As similar heterogeneity across studies was not seen in other analyses of ROH within consortia [Keller et al., 2012; McQuillan et al., 2012], the significant heterogeneity in our results suggest that MDD is particularly sensitive to differing demographics in the ascertainment of cases and controls and this may present a problem to genome-wide polygenic approaches such as ROH. Certainly no strong evidence for a recessive model was apparent, supporting the view of MDD being under weaker negative selection than other psychiatric disorders.

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Collaborators:

Michael R	Barnes	GlaxoSmithKline	UK
Thomas	Bettecken	Max Planck Institute of Psychiatry	Germany
Elisabeth B	Binder	Max Planck Institute of Psychiatry	Germany
Douglas HR	Blackwood	University of Edinburgh	UK
Dorret I	Boomsma	VU University, Amsterdam	The Netherlands
Gerome	Breen	Institute of Psychiatry, King's College London	UK
René	Breuer	Central Inst Mental Health, University of Heidelberg	Germany
Enda M	Byrne	Queensland Institute of Medical Research	Australia
Sven	Cichon	University of Bonn	Germany
William H	Coryell	University of Iowa	USA
Nick	Craddock	Cardiff University	UK
Ian W	Craig	Institute of Psychiatry, King's College London	UK
Darina	Czamara	Max Planck Institute of Psychiatry	Germany
Eco J	De Geus	VU University, Amsterdam	The Netherlands
Franziska	Degenhardt	University of Bonn	Germany
Anne E	Farmer	Institute of Psychiatry, King's College London	UK
Josef	Frank	Central Inst Mental Health, University of Heidelberg	Germany
Scott D	Gordon	Queensland Institute of Medical Research	Australia
Magdalena	Gross	University of Bonn	Germany
Steven P	Hamilton	University of California, San Francisco	USA
Andrew C	Heath	Washington University, St Louis	USA
Anjali K	Henders	Queensland Institute of Medical Research	Australia
Stefan	Herms	University of Bonn	Germany
Ian B	Hickie	University of Sydney, Sydney	Australia
Susanne	Hoefels	University of Bonn	Germany
Florian	Holsboer	Max Planck Institute of Psychiatry	Germany
Witte J	Hoogendijk	Erasmus Medical Center	The Netherlands
Jouke Jan	Hottenga	VU University, Amsterdam	The Netherlands
Marcus	Ising	Max Planck Institute of Psychiatry	Germany
Ian	Jones	Cardiff University	UK
Lisa	Jones	University of Birmingham	UK
Tzeng	Jung-Ying	North Carolina State University	USA
James A	Knowles	University of Southern California	USA
Martin A	Kohli	Max Planck Institute of Psychiatry	Germany
Ania	Korszun	Queen Mary University of London	UK
William B	Lawson	Howard University	USA
Douglas F	Levinson	Stanford University	USA
Cathryn M	Lewis	Institute of Psychiatry, King's College London	UK
Danyu	Lin	University of North Carolina	USA
Susanne	Lucae	Max Planck Institute of Psychiatry	Germany
Donald	MacIntyre	University of Edinburgh	UK
Pamela AF	Madden	Washington University, St Louis	USA
Wolfgang	Maier	University of Bonn	Germany
Nicholas G	Martin	Queensland Institute of Medical Research	Australia
Manuel	Mattheisen	University of Bonn	Germany
Patrick J	McGrath	Columbia University	USA
Peter	McGuffin	Institute of Psychiatry, King's College London	UK
Andrew	McIntosh	University of Edinburgh	UK
Alan	McLean	University of Edinburgh	UK
Christel M	Middeldorp	VU University, Amsterdam	The Netherlands
Lefkos	Middleton	Imperial College	UK
Grant M	Montgomery	Queensland Institute of Medical Research	Australia
Pierandrea	Muglia	GlaxoSmithKline	Italy
Bertram	Müller-Myhsok	Max Planck Institute of Psychiatry	Germany
Benjamin	Neale	Harvard University/Broad Institute	USA
Markus M	Noethen	University of Bonn	Germany
Willem A	Nolen	Groningen University Medical Center	The Netherlands
Dale R	Nyholt	Queensland Institute of Medical Research	Australia

Brenda P	Penninx	VU University Medical Center, Amsterdam	The Netherlands
Michele L	Pergadia	Washington University, St Louis	USA
James B	Potash	University of Iowa	USA
Marcella	Rietschel	Central Inst Mental Health, University of Heidelberg	Germany
Stephan	Ripke	Harvard University/Broad Institute	USA
William A	Scheftner	Rush University Medical Center	USA
Thomas G	Schulze	University of Goettingen	USA
Jianxin	Shi	National Cancer Institute	USA
Stanley I	Shyn	University of Washington	USA
Susan L	Slager	Mayo Clinic	USA
Johannes H	Smit	VU University Medical Center, Amsterdam	The Netherlands
Michael	Steffens	University of Bonn	Germany
Patrick F	Sullivan	University of North Carolina	USA
Federica	Tozzi	GlaxoSmithKline	Italy
Jens	Treutlein	Central Inst Mental Health, University of Heidelberg	Germany
Manfred	Uhr	Max Planck Institute of Psychiatry	Germany
Edwin JCG	van den Oord	Virginia Commonwealth University	USA
Gerard	VU University Medical Center, Amsterdam	The Netherlands	
Myrna M	Weissman	Columbia University	USA
Gonneke	Willemsen	VU University, Amsterdam	The Netherlands
Naomi R	Wray	The University of Queensland	Australia
Frans G	Zitman	Leiden University Medical Center, Leiden	The Netherlands

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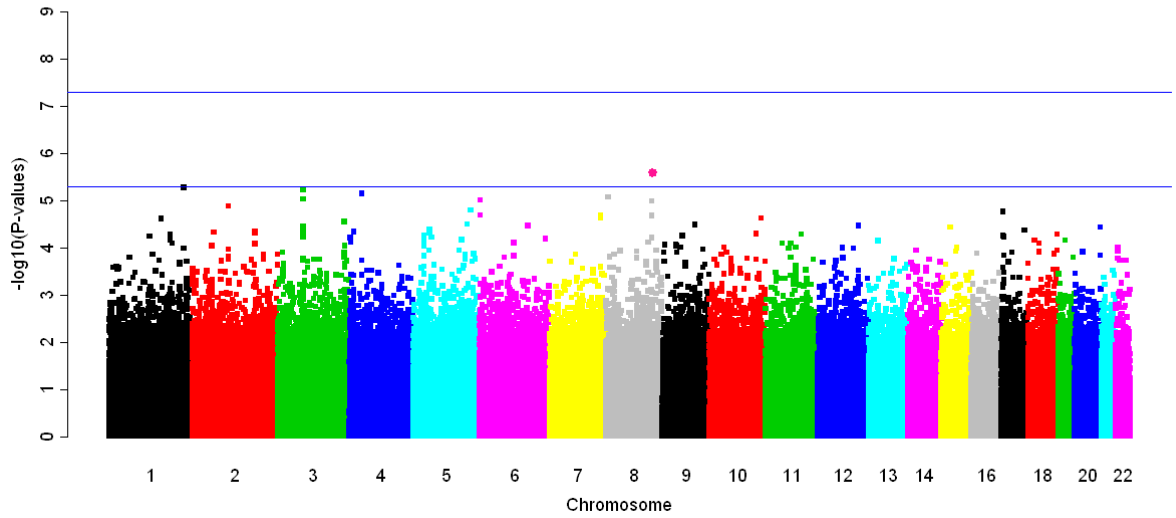
SUPPORTING INFORMATION

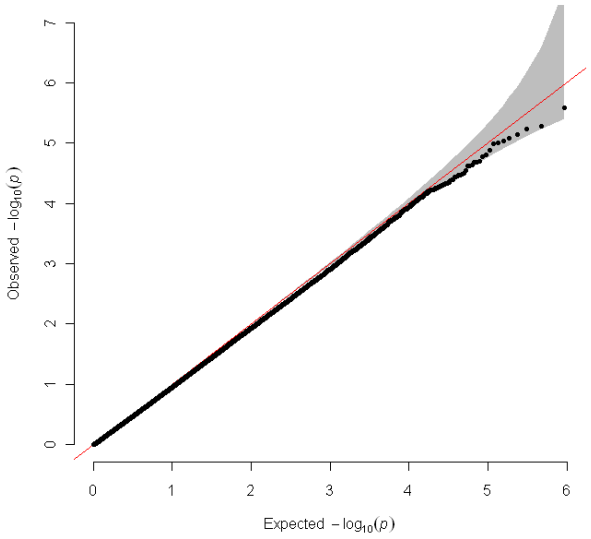
Additional supporting information may be found in the online version of this article at the publisher's web-site.

Supplementary materials

Supplementary Figure 1: Manhattan plot from p-values of genome-wide association analysis of major depression under a recessive model.



Supplementary Figure 2: QQ plot of p-values from genome-wide association analysis of major depression under a recessive model.



Supplementary Table 1:

Predictor	R^2	F_{ROH} increased in:	p-value
Percentage of recurrent cases	0.01	Controls	0.84
German study binary variable	0.58	Cases	0.02
US study binary variable	0.41	Controls	0.06
Australian study binary variable	0.04	Controls	0.59
Binary variable for if study was genotyped on Illumina	0.45	Cases	0.05
Binary variable for if copy number variant probes were available on the genotyping platform	0.01	Controls	0.79
Mean F_{ROH}	0.18	Cases	0.25
Mean genome-wide homozygosity	0.01	Controls	0.77

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The interaction between child maltreatment, adult stressful life events and the 5-*HTTLPR* in major depression

Robert A. Power [a,*], Lucy Lecky-Thompson [a], Helen L. Fisher [a], Sarah Cohen-Woods [a], Georgina M. Hosang [a], Rudolf Uher [a,b], Georgia Powell-Smith [a], Robert Keers [a,c], Maria Tropeano [a], Ania Korszun [c], Lisa Jones [d], Ian Jones [e], Michael J. Owen [e], Nick Craddock [e], Ian W. Craig [a], Anne E. Farmer [a], Peter McGuffin [a]

[a] MRC Social, Genetic and Developmental Psychiatry Centre, Institute of Psychiatry, King's College London, London, UK
[b] Department of Psychiatry, Dalhousie University, Halifax, NS, Canada
[c] Barts and The London School of Medicine and Dentistry, Queen Mary University of London, Centre for Psychiatry, London, UK
[d] Department of Psychiatry, School of Clinical & Experimental Medicine, University of Birmingham, Birmingham, UK
[e] MRC Centre for Neuropsychiatric Genetics and Genomics, Institute of Psychological Medicine and Clinical Neurosciences, Cardiff University, UK

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ABSTRACT

Both childhood maltreatment and adult stressful life events are established risk factors for the onset of depression in adulthood. However, the interaction between them can be viewed through two conflicting frameworks. Under a mismatch hypothesis stressful childhoods allow 'adaptive programming' for a stressful adulthood and so can be protective. Only when childhood and adulthood do not match is there a risk of behavioural problems. Alternatively, under the cumulative stress hypothesis we expect increased risk with each additional stressor. It has also been suggested that an individual's genetic background may determine the extent they undergo adaptive programming, and so which of these two hypotheses is relevant. In this study we test for an interaction between exposure to childhood maltreatment and adult stressful life events in a retrospective sample of 455 individuals, using major depression as the outcome. We also test whether this interaction differs by genotype at the 5-*HTTLPR*, a candidate for an individual's plasticity to adaptive programming.

Early maltreatment and stressful life events in adulthood interacted to produce increased risk for depression over each individually ($p = 0.055$). This supports the cumulative stress hypothesis over the mismatch hypothesis, at least with respect to severe environmental risk factors. This effect was not altered by 5-*HTTLPR* allele, suggesting there was no difference by genotype in adaptive programming to these events. We suggest that the apparent additional vulnerability to stressful events of those who have experienced maltreatment has clinical relevance, highlighting the importance of providing support beyond the immediate aftermath of maltreatment into adulthood.

1. Introduction

Child maltreatment (such as abuse or neglect) has long been associated with increased risk of psychopathology in adulthood, including depression (Edwards et al., 2003; Mazure, 1998; Windle et al., 1995), and presents a major social burden. Adult stressful life events (e.g. bereavement and divorce) also predict risk for the onset of depression (Brown and Harris, 1989; Monroe et al., 1999; Paykel et al., 1980) and triggering depressive episodes (Hosang et al., 2012). However, the interaction between these two types of adversity is far from simple. In a recent review of the topic, Nederhof and Schmidt (2012) described two conflicting models, the cumulative stress hypothesis and mismatch hypothesis, for explaining the interactions between early and adult stressors, and proposed a study design for differentiating them.

The cumulative stress hypothesis states that disease risk increases as adversity accumulates through life, the more traditional view for which there is considerable evidence e.g. (Brown et al., 2008; Kendler et al., 2004; McLaughlin et al., 2010). The mismatch hypothesis stipulates that individuals are primed to undergo "adaptive phenotypic programming" during development, in order to best match their adult phenotype with the predicted

* Corresponding author.
E-mail address: robert.r.power@kcl.ac.uk (R.A. Power).

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environment (Frankenhuis and Del Giudice, 2012). In this case, a stressful childhood leads to developmental changes to prepare for a stressful adulthood. In contrast to the cumulative risk hypothesis, in the mismatch hypothesis individuals are more likely to experience disease if the early environment is different from the later adult environment; when the predicted programming and experienced environment are not in agreement. Here, one might imagine an individual adapted for low levels of stress responding poorly to adversity. Alternatively, an individual adapted for high levels of stress but encountering none may experience an apparent excess of anxiety or paranoia relative to their stress free environment. A recent review of animal models testing the mismatch hypothesis highlighted numerous examples in agreement with the model, but also many that were not (Schmidt, 2012).

One potentially confounding factor is that individuals have been predicted to differ genetically in their ability to experience adaptive programming and susceptibility to the environment (Belsky et al., 2009). Those who are genetically very susceptible to programming are more likely to follow the mismatch hypothesis (so called 'plastic' individuals), whereas those with no genetic susceptibility to programming do not undergo the initial adaptation and so the adverse effects of stress will accumulate. An individual's burden of 'plasticity polymorphisms' may therefore determine whether they respond according to the mismatch or the cumulative hypothesis, and may explain some of the conflicting results to date. One such genetic plasticity factor is a polymorphism within the promoter region of the serotonin transporter gene (5-*HTTLPR*), a candidate for plasticity suggested by Nederhof and Schmidt and previously found to interact with environmental risk factors for depression (Caspi et al., 2003; Eley et al., 2004; Fisher et al., 2013; Karg et al., 2011; Schmidt, 2012; Taylor et al., 2006; Uher et al., 2011).

Here we seek to follow Nederhof and Schmidt's (2012) proposed study design to test the cumulative risk model and the mismatch model in explaining major depression, including an attempt to incorporate an individual's genetic susceptibility to adaptive programming. We use reported levels of childhood maltreatment and stressful life events prior to interview/depressive episode as markers for early and adult stressors, respectively. As well as looking at their interaction in the whole sample, we also test stratifying by genotype at the 5-*HTTLPR* which, as mentioned, has been explored as a candidate for gene–environment interactions including within this sample (Fisher et al., 2013, 2012). To our knowledge, this will be the first human study incorporating genotypic data into a test of the mismatch hypothesis. A finding in support of either the cumulative stress or mismatch hypothesis has relevance to how individuals who have experienced childhood stress are likely to be affected by adult stress and risk of depression.

2. Methods

2.1. Participants

Individuals with recurrent unipolar depression and healthy controls were drawn from the Cardiff and London sites of the Depression Case–Control (DeCC) multi-centre study (Cohen-Woods et al., 2009). This study was approved by the local University and NHS Ethics Committees at each site and all participants provided written informed consent. Patients were identified through psychiatric clinics, hospitals, general medical practitioner surgeries, and media advertisements. Patients must have experienced at least two episodes of depression of at least moderate severity, separated by 2 or more months of remission, as defined by DSM-IV (American Psychiatric Association, 1994) and/or ICD-10 (World Health Organisation, 1993). All participants were aged 18 or over and had parents and grandparents of white European origin. Exclusion criteria were a history of mania or hypomania, mood-incongruent psychosis, and a first or second- degree relative with bipolar or psychotic disorder. Controls were recruited through UK general medical practices and excluded if they had a personal or first-degree relative with a history of any psychiatric disorder.

2.2. Measure of child maltreatment

Self-reported abuse or neglect during childhood, defined as until age 17, was recorded using the Childhood Trauma Questionnaire (CTQ) (Bernstein et al., 2003). The CTQ is a 28 item questionnaire widely used in clinical and general population samples and has good psychometric properties (Bernstein et al., 2003; Scher et al., 2004). Here we used a dichotomous variable to differentiate those who had experienced abuse or neglect from those who had not. This used a cut-off score of 8 or more for the sexual abuse, physical abuse or neglect subscales; a cut-off of 10 for emotional abuse; and a cut-off of 15 for the emotional neglect subscale. If an individual's scores exceeded any of these cut-offs, they were deemed to have experienced childhood maltreatment (Fisher et al., 2013).

2.3. Measure of stressful life events

The List of Threatening Experiences Questionnaire (LTE-Q) (Brugha et al., 1985) was used to record 11 stressful life events that occurred 6 months before cases' most severe episode of depression or 6 months prior to interview for controls (see Hosang et al., 2010). Each event was rated on a scale of zero (not present) to 3 (severe) and in this analysis, only severe events were counted. As in the measure of childhood maltreatment, the measure of stressful life events was dichotomized into those with any severe events and those with none (Fisher et al., 2012).

2.4. Genotyping

A 25 ml sample of whole blood was collected from cases at the time of interview and six cheek swabs were obtained from controls by mail. Polymerase chain reaction (PCR) was performed on the samples to amplify a 419 base-pair product for the l-allele (16-repeat) and a 375 base-pair product for the s-allele (14-repeat) of the 5-*HTTLPR* (Gelernter et al., 1997). The primer sequences were TGCCAGCACCTAACCCCTAATGT (forward) and GGACCGCAAGGTGG GCGGGA (reverse). The products were run on 2.5–3% agarose gel at 100 mV for 1 h. Genotyping was conducted blind to depression status and life events, and is described in previous publications (Fisher et al., 2013).

2.5. Analysis

In a logistic regression model we tested for each combination of stressors (childhood maltreatment only, adult stressors only, or both) against those who had no stressors, with depression as the outcome. We also tested whether an additive interaction between the dichotomous variables for childhood maltreatment and adult stressful life events was predictive of depression, using a binomial regression. Analyses were corrected for sex and age at assessment. Lastly, we tested to see if the direction of effect for these analyses differed depending on genotype at the 5-*HTTLPR*, using a dominant model for carriers of the short allele. We tested for normality of residuals and heteroscedasticity, and to account for the latter in some regressions we checked the results were consistent when robust standard errors were used. All analyses were carried out in STATA (StataCorp., 2011).

Table 1
Prevalence of environmental stressors and genotypes at 5-*HTTLPR* amongst cases and controls.

	N	Percentage female	Mean age at assessment (SE)	Mean age for adult SLE[a] (SE)	Mean child maltreatment score (SE)	Number reporting childhood maltreatment (%)	Mean adult SLE[a] score (SE)	Number reporting adult SLE[a] (%)	Number of 5-*HTTLPR* short allele carriers (%)
Cases	227	71.80%	45.4 (0.84)	37.9 (0.88)	46.0 (1.1)	184 (81%)	1.17 (0.08)	146 (65%)	157 (69%)
Controls	228	60.00%	47.2 (0.62)	47.2 (0.62)	33.0 (0 0.61)	73 (32%)	0.69 (0.07)	106 (46%)	146 (64%)

[a] SLE = stressful life events.

3. Results

The total number of individuals with phenotype and genotype data was 455, made up of 227 unipolar depression cases (71.8% female, mean age = 37.9, age range = 8–72) and 228 controls (60.1% female, mean age = 47.2, age range = 25–62). Of these, 257 individuals reported childhood maltreatment (71% cases) and 253 reported adult stressful life events prior to interview/worst episode of depression (58% cases) (see Table 1). The correlation between childhood maltreatment and adult stressful life events was small but significant ($r = 0.16$, $p = 0.0005$). The odds ratio for the prediction of adult stressful life events by childhood maltreatment in the whole sample was 1.74 ($p = 0.006$), but was only significant in cases (OR = 2.4, $p = 0.01$). The short allele of the 5-*HTTLLPR* had a frequency of 42.6%, and was in Hardy–Weinberg Equilibrium ($p = 0.66$). 5-*HTTLLPR* genotype was not associated with depression status.

Child maltreatment was associated with depression in those with and without stressful life events, but stressful life events were only linked to elevated rates of depression when combined with child maltreatment (see Table 2). Moreover, the odds were much greater when individuals reported exposure to both child and adult stressors than for child maltreatment alone, increasing from an OR of 5.1–12.4 (Table 2). There was tentative evidence of an interaction effect between childhood maltreatment and adult stressful life events (see Table 2, $p = 0.055$). The same effect size for this interaction was seen in both those who did and did not carry a copy of the short allele at the 5-*HTTLPR*, suggesting no difference in propensity towards either mismatch or cumulative hypothesis based on genotype. There was a much greater effect of child maltreatment on the risk of depression in those individuals carrying the short allele (from OR of 7.8–15.8), reflecting the gene–environment interaction of risk seen previously in this sample (Fisher et al., 2013).

4. Discussion

Our findings provide clear evidence against the mismatch hypothesis for major depression with respect to the environmental stressors examined here, and supports a view of cumulative risk from repeated adversity during an individual's lifespan. Furthermore, the results indicate an interaction effect, where adult stressful life events were only associated with depression in those individuals who had experienced childhood maltreatment. While we focused only on one candidate genetic polymorphism in the serotonin transporter gene (5-*HTTLPR*), we did not find evidence for an underlying genetic susceptibility to adaptive programming in response to childhood maltreatment and future stressful events.

The major limitation of this study is the measurements of early and late stressful events. Brief self-report questionnaires were utilised to retrospectively assess stressors which may have biased the information obtained. However, high concordances between the self-reported LTE-Q used here and more detailed measures of adult life events such as the Life Events and Difficulties Schedule have previously been reported (Hosang et al., 2010). Self-reporting of childhood maltreatment has also shown to agree with more objective measures in the form of hospital records (McGuffin et al., 1986). However the real issue is the severity of the measures of environment. Childhood maltreatment, as measured here, presents a very severe negative experience. It is likely that adaptive programming only occurs with respect to milder measures of childhood stress which are less damaging to an individual and allow them to recover. Unfortunately no such measures were available here, and we hope this study will promote the importance of detailed environmental phenotyping beyond the most severe risk factors. With regards to adult stressful life events we were limited to those events prior to the most severe depressive episode, and it is possible that we might have found different results if we had been able to use events prior to the initial onset of depression. Only for a very small proportion of this sample was the worst episode also the first episode (~7%), prohibiting such an analysis. While not ideal, the measure of events prior to the most severe episode still captures an individual's sensitivity to environmental stressors relative to controls and so should not be dismissed as uninformative. Lastly, this study focused solely on severe recurrent depression as an outcome and it is possible that the mismatch framework may be operating for other behavioural phenotypes.

Overall a better understanding of how risk factors interact for disorders such as depression is important in guiding the treatment and management of affected individuals. This is especially true where there is the possibility of adaptive programming, and so the possibility of environmental interventions. Here we have shown novel findings in support of the cumulative stress hypothesis explaining risk for depression over the mismatch hypothesis, and highlighted the increased risk of those individuals who have suffered childhood maltreatment. This also highlights the need to

Table 2
Odds ratios (OR) and *p*-values for depression when comparing those who have experienced no stressors ($n = 106$) to those who have experienced child maltreatment only ($n = 96$), adult stressful life events only ($n = 92$), or both ($n = 161$), and an interaction term for the two variables. Results also shown by 5-*HTTLPR* genotype in a dominant model for the short allele.

Adversity	Total sample		Long allele homozygotes ($n = 152$)		Short allele carriers ($n = 302$)	
	Or (95% CI)	*p*-value	Or (95% CI)	*p*-value	Or (95% CI)	*p*-value
Only adult	1.01 (0.50–2.04)	0.98	1.02 (0.33–3.18)	0.97	0.94 (0.37–2.36)	0.89
Only child	5.05 (2.56–9.94)	<0.001	2.7 (0.88–8.27)	0.08	7.3 (3.02–17.64)	<0.001
Both	12.41 (6.53–23.60)	<0.001	7.82 (2.62–23.23)	<0.001	15.75 (7.01–35.37)	<0.001
Interaction	0.92 (−0.02 to 1.88)	0.055	0.99 (−0.57 to 2.56)	0.21	0.93 (−0.28 to 2.14)	0.13

CI, confidence interval.

equip such individuals with the tools and support to deal with future stressful events beyond the immediate aftermath of such maltreatment, and into adulthood.

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Contributors

Robert A. Power and Lucy Lecky-Thompson were involved in the writing of the manuscript and proposed the analysis. Helen L. Fisher, Sarah Cohen-Woods, Georgina M. Hosang, Rudolf Uher, Georgia Powell-Smith, Robert Keers and Maria Tropeano were involved in the analysis of both environmental and genetic data. Ania Korszun, Lisa Jones, Ian Jones, Mike Owen, Nick Craddock, Ian W. Craig, Anne E. Farmer and Peter McGuffin were involved in the overall study design, selection of measures, and recruitment of individuals.

Conflicts of interest

Anne Farmer and Peter McGuffin have received consultancy fees and honoraria for participating in expert panels from pharmaceutical companies including Lundbeck and GlaxoSmithKline. All other authors declare that they have no conflicts of interest. Georgina Hosang has provided a sponsored talk for Bristol-Myers Squibb.

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RESEARCH ARTICLE

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Genome-Wide Association Analysis Accounting for Environmental Factors Through Propensity-Score Matching: Application to Stressful Live Events in Major Depressive Disorder

Robert A. Power,[1]* Sarah Cohen-Woods,[1,2] Mandy Y. Ng,[1] Amy W. Butler,[1,3] Nick Craddock,[4] Ania Korszun,[5] Lisa Jones,[6] Ian Jones,[4] Michael Gill,[7] John P. Rice,[8] Wolfgang Maier,[9] Astrid Zobel,[9] Ole Mors,[10] Anna Placentino,[11] Marcella Rietschel,[12] Katherine J. Aitchison,[1] Federica Tozzi,[13] Pierandrea Muglia,[13,14,15] Gerome Breen,[1,16] Anne E. Farmer,[1] Peter McGuffin,[1] Cathryn M. Lewis,[1] and Rudolf Uher[1,17]

[1]MRC Social Genetic and Developmental Psychiatry Centre, Institute of Psychiatry, King's College London, London, United Kingdom

[2]Discipline of Psychiatry, School of Medicine, University of Adelaide, Adelaide, SA 5005, Australia

[3]Department of Psychiatry, University of Hong Kong, Hong Kong, Special Administrative Region, China

[4]MRC Centre for Neuropsychiatric Genetics and Genomics, Neuroscience and Mental Health Research Institute, Cardiff University, Cardiff, United Kingdom

[5]Barts and The London Medical School, Queen Mary University of London, London, United Kingdom

[6]Department of Psychiatry, School of Clinical & Experimental Medicine, University of Birmingham, Birmingham, United Kingdom

[7]Department of Psychiatry, Trinity Centre for Health Science, Dublin, Ireland

[8]Department of Psychiatry, Washington University, St. Louis, Missouri

[9]Department of Psychiatry, University of Bonn, Bonn, Germany

[10]Centre for Psychiatric Research, Aarhus University Hospital, Risskov, Denmark

[11]UOP 23-Department of Mental Health, Spedali Civili Hospital and Biological Psychiatry Unit-Centro San Giovanni di Dio, FBF, Brescia, Italy

[12]Department of Genetic Epidemiology in Psychiatry, Central Institute of Mental Health, Medical Faculty Mannheim, University of Heidelberg, Mannheim, Germany

[13]GlaxoSmithKline Research & Development, Verona, Italy

[14]Department of Psychiatry, University of Toronto, Toronto, Canada

[15]NeuroSearch A/S, Ballerup, Denmark

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*Correspondence to:
Robert A. Power, MRC Social, Genetic and Developmental Psychiatry Centre, Institute of Psychiatry, London SE5 8AF, United Kingdom.
E-mail: robert.r.power@kcl.ac.uk

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[16]NIHR Biomedical Research Centre for Mental Health, South London and Maudsley NHS Foundation Trust and Institute of Psychiatry, King's College London, London, United Kingdom

[17]Department of Psychiatry, Dalhousie University, Halifax, NS, Canada

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Stressful life events are an established trigger for depression and may contribute to the heterogeneity within genome-wide association analyses. With depression cases showing an excess of exposure to stressful events compared to controls, there is difficulty in distinguishing between "true" cases and a "normal" response to a stressful environment. This potential contamination of cases, and that from genetically at risk controls that have not yet experienced environmental triggers for onset, may reduce the power of studies to detect causal variants. In the RADIANT sample of 3,690 European individuals, we used propensity score matching to pair cases and controls on exposure to stressful life events. In 805 case–control pairs matched on stressful life event, we tested the influence of 457,670 common genetic variants on the propensity to depression under comparable level of adversity with a sign test. While this analysis produced no significant findings after genome-wide correction for multiple testing, we outline a novel methodology and perspective for providing environmental context in genetic studies. We recommend contextualizing depression by incorporating environmental exposure into genome-wide analyses as a complementary approach to testing gene-environment interactions. Possible explanations for negative findings include a lack of statistical power due to small sample size and conditional effects, resulting from the low rate of adequate matching. Our findings underscore the importance of collecting information on environmental risk factors in studies of depression and other complex phenotypes, so that sufficient sample sizes are available to investigate their effect in genome-wide association analysis.

Key words: depression; propensity score matching; stressful life events; genome-wide association studies; gene–environment interactions

INTRODUCTION

Depression is a common disorder with a complex etiology. There is a substantial heritable component to risk, although heritability estimates vary across severity and clinical subgroups [McGuffin et al., 1996; Sullivan et al., 2000; ten Doesschate et al., 2010; Lubke et al., 2012]. Recent genome-wide association studies have largely been unsuccessful in detecting genetic variants associated with depression with only one replicated finding [Ising et al., 2009; Sullivan et al., 2009; Lewis et al., 2010; Kohli et al., 2011; Shi et al., 2011; Major Depressive Disorder Working Group of the Psychiatric GWAS Consortium, 2012], in contrast to the relative success in other psychiatric and medical disorders [Ripke et al., 2011; Sklar et al., 2011]. One potential source of heterogeneity for genome-wide association studies is the strong role of environmental adversity in the etiology of depression.

Stressful life events (SLEs) such as illness, unemployment, or loss of a close relative or friend, have been strongly associated with the onset of depression [Paykel et al., 1980; Brown and Harris, 1989; Monroe et al., 1999]. Previously, SLEs have been exploited in the study of gene–environment interactions (G × E), which looked for genetic risk that varies according to the level of stress exposure [Caspi et al., 2003; Uher and McGuffin, 2008; Risch et al., 2009; Fisher et al., 2012]. However, G × E studies have low statistical power to capture the effect of genetic variants that would be associated with depression but whose effects may be overwhelmed at extreme levels of environmental exposure, or if the exposure to environmental factors is uneven in the population [Caspi et al., 2010]. Aside from statistical considerations, it is also relevant to put depressive episodes into the context of an individual's environment. Horwitz and Wakefield [2007] have argued that current classifications of major depression which, excluding bereavement, do not properly account for environmental factors in diagnosis. They suggest that ignoring the environmental context of depression risks medicalizing a rational response to stressful experiences. This may contribute to heterogeneity within cases by including those individuals reacting "normally" in response to their environment. On the other hand, restricting to those controls with exposure to environmental triggers for depression helps to screen out those genetically predisposed to the disorder that may develop it later in life when environment changes. For these reasons, G × E studies need to be complemented with a search for genetic effects that are associated with depression among individuals living in similar environments.

Psychiatric research is not the only area where matching or accounting for environmental measures in genome-wide association analyses is important, and so several methods have been proposed. An early example of this was the association between type 2 diabetes and *FTO*, a gene also associated with body mass index [Wellcome Trust Case Control Consortium, 2007]. The association with type 2 diabetes generally disappears in studies where only low body mass index cases were recruited, but was

apparent in those where cases were un-stratified [Frayling et al., 2007; Saxena et al., 2007; Freathy et al., 2008]. Several methodologies have been suggested for genome–environment wide interaction studies, or GEWIS, to combat the previously mentioned issues of low power in such studies [Thomas, 2010], but few focus on formal matching of samples based on environmental risk.

In order to disentangle environmental risk that might contaminate genetic studies, this article describes a novel method to incorporate environmental exposure variables into GWAS. Here we use propensity score matching to pair cases and controls on SLEs before performing a sign test across pairs to look for association of affection status with each single nucleotide polymorphism (SNP). As cases and controls differ in environmental exposure, this method reduces the number of controls with few environmental risk factors and decreases the number of cases with large environmental risk burdens. This allows the comparison of like with like between cases and controls that have experienced similar levels of environmental stress but developed contrasting outcomes, and potentially reduces heterogeneity within the sample.

METHODS

Sample

We used several studies that recruited participants in the same or similar manner and which are jointly known as RADIANT. The RADIANT study comprises three cohorts of depression cases: a case–control study, DeCC [Gaysina et al., 2008]; an affected sibling pair linkage study, DeNT [Farmer et al., 2004]; and a pharmacogenetic study, GENDEP [Uher et al., 2009]. DeCC cases were ascertained from three UK clinical centers (London, Cardiff, and Birmingham). Cases from DeNT and GENDEP were ascertained more widely from clinical centers across Europe for GENDEP (London, Brussels, Mannheim, Bonn, Brescia, Aarhus, Ljubljana, Poznan, and Zagreb) and from Europe and the US for DeNT (Birmingham, Cardiff, St. Louis, Aarhus, London, Bonn, Dublin, and Lausanne). Controls from DeCC and the bipolar case–control study, BACCS [Gaysina et al., 2009], were collected in the UK, and screened for absence of any psychiatric disorder. All DeCC and DeNT cases and 60.4% of GENDEP cases were recurrent (2 or more episodes of MDD). Genotyping was performed on the Illumina 610K beadarray. Individuals were excluded if their genotypic data showed a missing rate >1%, abnormal heterozygosity, a sex assignment that conflicted with phenotypic data, if they were related (up to second degree) with other study members, or were of non-European ancestry. SNPs with a low minor allele frequency (<1%) or showing departure from Hardy–Weinberg equilibrium ($P < 1 \times 10^{-5}$) were excluded. No imputation method was used. Full details of genotyping methods and quality control procedures for a genome-wide study of depression in the UK have been published [Lewis et al., 2010]. All study participants provided informed consent, and ethical approval was obtained from relevant institutional review boards. In all analyses we restricted to individuals recruited in Western European centers to reduce both potential genetic and reporting differences.

Stressful Life Event data

All studies included the List of Threatening Experiences Questionnaire [LTE-Q; Brugha et al., 1985; Brugha and Cragg, 1990] for both cases and controls, with cases and controls reporting for 6 months before interview and cases having additional information for 6 months before worst episode. For the purposes of this analysis only data for events prior to worst episode was used for cases. The LTE-Q was administered to all participants during their interview; this entailed asking whether they experienced an event, confirming the event occurred during the specified index period and obtaining some contextual information to establish that the reference event fulfilled the classification of the items listed on this instrument. For example, the respondent reporting a cold or flu would not be included under the "personal illness" category of event (insufficiently severe illness). While the original LTE-Q consists of 12 events, two of these were combined into a single item for the present study (these were: "did you have a separation due to marital difficulties?" and "did you break off a steady relationship?" which were combined into "did you have a separation due to marital difficulties or break off a steady relationship?"). Childbirth was added as an additional stressful life event. These events had previously been found to be associated with depression status, both as a cumulative score and individually [Hosang et al., 2010, 2012]. Each SLE was rated on a four-point scale severity, with a rating of not present, mild, moderate, or severe.

Matching

Cases and controls were first matched on ancestry-informative principal components (PCs) to deal with population stratification, and then independently matched on SLEs. Both steps were performed within STATA MP [StataCorp., 2011]. The first step used the CEM package [Iacus et al., 2009], which performs coarsened exact matching creating strata based on the desired variables. This program was developed to prune individuals that lacked closely matching pairs before using propensity scores to identify specific matches. Here it was used to create strata based on the first two PCs. Within each stratum, propensity score matching was used to match cases and controls based on each of the 12 items from the modified LTE-Q. This generated a probability of each individual being either a case or a control, based on these 12 SLEs. Affected individuals were then matched to an unaffected individual based on propensity scores using nearest neighbor matching in the psmatch2 package [Leuven and Sianesi, 2003]. A threshold difference in propensity score of 0.2 within each pair was imposed to restrict analysis to the best matched pairs, standard for this type of analysis [Guo and Fraser, 2009].

Association Testing: Logistic and Allelic Sign Test

A non-parametric test was performed for each SNP to assess whether cases carried more (or fewer) rare alleles than controls. For each SNP, the number of cases carrying more rare alleles than their paired control was tabulated, giving a total of n+ pairs. This was compared to the number of pairs where cases carried fewer rare

alleles than the paired controls (n−). Departure from the null hypothesis of an equal probability that the paired cases and controls carried more rare alleles was tested using a sign test (expected proportion of cases having more rare alleles than controls = (n+ + n−)/2).

For comparison, an unconditional logistic regression was performed within PLINK [Purcell et al., 2007] with analysis restricted to those cases and controls that were matched on both PCs and SLEs. In the logistic regression analysis, no PCs were used as covariates to assess the effect of matching on population stratification. To correct for multiple testing, a threshold *P*-value of 5E−8 was used to identify associations of genome-wide significance, with $P = 5E{-}6$ used as a cut off for suggestive significance.

RESULTS

Propensity Score Matching

Analysis of the first two principal components produced 86 strata. Pruning to only those strata with at least one case and control resulted in 3,497 individuals in 60 strata remaining from the initial 3,690. The number of individuals in each stratum ranged from 2 to 435, with a median number of 25 individuals. Within these individuals, the average score for stressful life events was 3.8 (SD 3.9) for cases and 1.3 (SD 1.8) for controls. Propensity score matching resulted in 805 pairs of cases and controls passing our requirements for thresholds on principal components and stressful life events. Within these individuals, both total number of SLEs and each individual SLE were no longer significantly associated with affected status ($P > 0.05$). The mean difference in propensity score was 0.022 (SE 0.0016). After matching, the average score for life events was 1.1 (SD 1.8) for cases and 1.1 (SD 1.7) for controls, showing that on average those cases with a very high number of environmental risk factors had not been matched and so removed. This matched cases with the highest genetic and lowest environmental component to controls (Table I).

Association Analysis

After quality control, 457,670 SNPs remained. Genome-wide analysis using a sign test of allele direction across all pairs resulted in a lambda value of 1.01, suggesting that matching within strata based on principal components effectively dealt with population stratification. There was a clear deflation in the number of SNPs with highly significant associations compared to the expected distribution of test statistics (Fig. 1), which may have been due to overcorrecting by matching on both SLEs and principal components. The sign test revealed no SNPs associated at suggestive or genome-wide significance (Fig. 2). Logistic regression was also performed in the restricted sample of matched individuals, without pairing. Again, no principal components were used and again after previous matching on PCs there was no evidence of population stratification (lambda = 1.02, Fig. 1). Two SNPs reached suggestive significance in this analysis (see Table II). The first was rs8050326 on the q24 arm of chromosome 16 near the gene *IRF8* ($P = 2.6 \times 10^{-7}$) which had been found in the original case–control analysis of this dataset. The other was rs11152166 on the q21 arm of chromosome 18 within the *CCBE1* gene ($P = 2.7 \times 10^{-6}$). Neither of these regions has been identified in depression studies outside of the primary analysis of this data. Correlation of the log *P* values from the sign test with those from the logistic regression was 0.25, suggesting they may have tested largely independent hypotheses with matching adding information to the analysis beyond simply pruning individuals.

TABLE I. Summary Statistics of Stressful Life Events by Affection Status

	Total sample		Matched sample	
Using % for events	**Cases**	**Controls**	**Cases**	**Controls**
N	2,626	1,064	805	805
Percentage female	71%	59%	71%	60%
Mean age[a] (SE)	37.8 (0.24)	41.5 (0.40)	38.29 (0.48)	41.9 (0.46)
Illness or injury of self	16.3	5.7	4.8	6.2
Illness or injury of other individual	17.5	15.0	12.3	12.9
Death of first degree relative	9.7	2.8	1.9	2.5
Death of other individual	12.3	15.3	9.9	10.2
Separation/divorce from partner	21.1	4.6	3.5	4.6
Conflict with close friend or family	24.1	7.6	6.0	7.9
Redundancy	8.5	1.9	1.0	1.9
Unemployment	8.6	3.6	1.7	2.6
Financial problems	13.8	3.8	2.9	3.5
Legal problems	3.0	1.0	0.4	0.6
Loss of valued item	5.9	5.4	2.5	3.3
Childbirth	7.0	0.8	0.4	0.7

[a]Age at interview for controls and age at worst episode for cases.

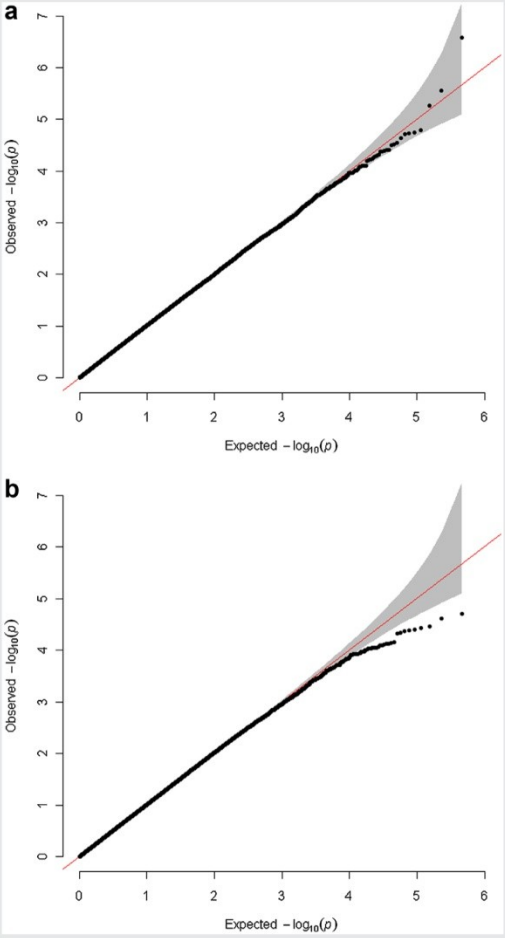

FIG 1. Comparison of QQ plots from genome-wide analysis of cases and controls matched on stressful life events for unpaired logistic regression (left, a) and paired sign test (right, b).

Figures 1 and 2 show a comparison of the QQ and Manhattan plots for these two analyses.

DISCUSSION

The role of environmental risk factors in depression is both important and complex. Differences between cases and controls in environmental risk could introduce a source of heterogeneity into genome-wide association studies for the disorder. Here we applied a novel approach to tackle this issue, restricting analysis to cases and controls matched for exposure to stressful life events. This is of particular importance as cases and controls differ in terms of exposure. While this is possibly partially due to reverse causation from traits like neuroticism that have been associated with both depression and increased reporting of negative events [Farmer et al., 2002], it also highlights the risk that differences in exposure to environmental risks may introduce heterogeneity into the sample. While our results did not provide any genome-wide significant results, the novel method for incorporating environmental data into GWAS may be more generally applicable.

The most likely explanation for the lack of positive findings here is a lack of power, considering the large sample sizes required to identify risk variants in genome-wide association studies. Propensity score matching on covariates that show a large difference between cases and controls, such as SLEs, will inevitably result in some individuals being unable to be matched. This reflects the large discrepancy between cases and controls on exposure to environmental adversity and illustrates the level of heterogeneity that may impair the power of GWAS of stress-related disorders. This method may be more applicable to studies where cases and controls have been ascertained to reduce differences in environment, or where the influence of covariates is believed to reduce power to a degree that is greater than that from the reduced sample size. This is plausible as it has been shown that power can be increased through conditioning cases and controls on the basis of clinical covariates [Zaitlen et al., 2012], while simply correcting for them can substantially reduce power unless the disorder is highly prevalent [Pirinen et al., 2012]. A further consideration in the merits of matching rather than covarying is how the covariate in question is distributed amongst cases and controls. If the distribution of a covariate does not overlap completely between cases and controls, as is true for higher burdens of SLEs, covarying out its effects becomes problematic.

An additional serious concern is that of an alternate framework for SLEs. It has been shown that the reporting of SLEs is itself heritable, including in the sample used for the present study [Plomin et al., 1990; Kendler and Baker, 2007; Power et al., 2012]. If this heritability overlaps considerably with the heritability of depression (plausible considering the association between depression and SLEs), then matching cases and controls on SLEs would increase their genetic similarity due to gene–environment correlation. Partial overlap between genetic factors contributing to depression and reported SLE has been reported, though only with respect to certain types of stressful life events [Thapar et al., 1998; Rice et al., 2003; Boardman et al., 2011]. Gene–environment correlation may have been problematic for another reason, as individuals were matched on both ancestry-informative principal components and SLEs. This may have led to over-correction, reducing the genetic differences between cases and controls. However, the presence of an underlying genetic component to SLEs only further strengthens the likelihood of genetically distinct sub-types of "environmental" and "biological" depression, and so the very heterogeneity this method was designed to remove. However, as only events in the previous 6 months were used, some environmental variance may have been left unaccounted for in our matching of cases and controls. Lastly, it is also possible our negative results are due to

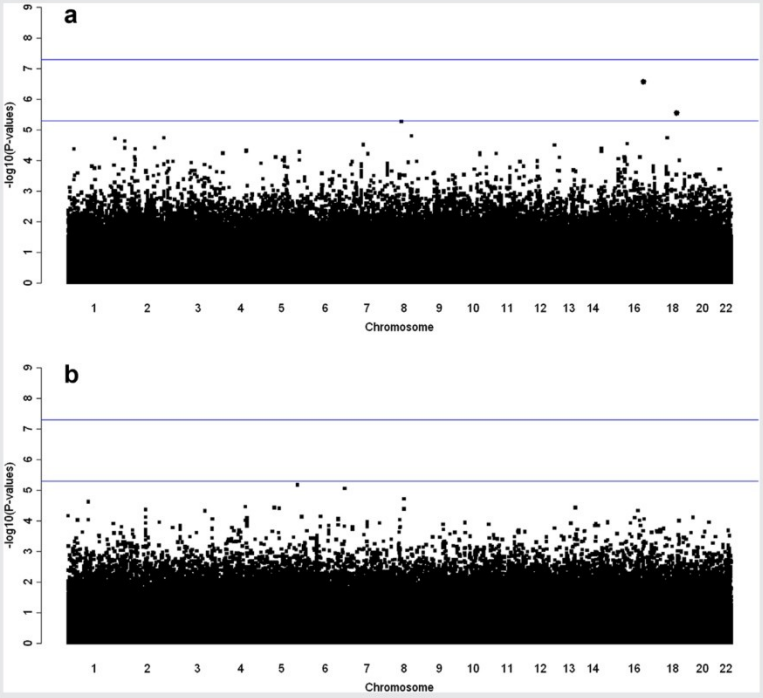

FIG 2. Comparison of Manhattan plots from genome-wide analysis of cases and controls matched on stressful life events for unpaired logistic regression (left, a) and paired sign test (right, b).

a small underlying genetic component of depression, though for a recurrent clinical cohort as examined here this should not be the case [McGuffin et al., 1996; Sullivan et al., 2000; Levinson, 2006]. As with any genome-wide association study it is possible that the causal variants are not tagged by those variants genotyped, for example, copy number variants or rare variants.

Our method of stratifying by principal components before matching on SLEs successfully removed evidence of population stratification in both the paired sign test and in the unpaired logistic regression analyses. However the main limitation of this study was clear, that the loss in power due to reduced sample size must be outweighed by the increased power from reduced heterogeneity. It's worth noting that our approach prunes sample size in proportion to the difference in environment between cases and controls, and so in proportion to the potential reduction of power this difference introduces. That there is such a large difference in SLEs between cases and controls identified here suggests the scale of potential heterogeneity introduced. We hope that by providing a detailed strategy for incorporating environmental factors into a GWAS will provide additional motivation to collect information on environmental adversity in future studies which may succeed in identifying genetic variants associated with depression and other stress-related disorder by accounting for environmental factors. This contrasts with recent large studies of psychiatric illness where the aim has

TABLE II. Top SNPs From Logistic Regression Analysis of Those Individuals Who Remained After Pruning for Principal Components and Stressful Life Events

CHR	SNP	BP	Effect allele	Frequency	OR	SE	L95	U95	*P*-value
16	rs8050326	8,46,61,643	A	0.44	1.45	0.072	1.26	1.67	2.64E−07
18	rs11152166	5,54,65,787	C	0.42	0.71	0.072	0.62	0.82	2.74E−06

been to maximize sample size, at the cost of standardized phenotype data [Purcell et al., 2007; Ripke et al., 2011; Major Depressive Disorder Working Group of the Psychiatric GWAS Consortium, 2012]. While this has succeeded with other psychiatric disorders, it has failed to yield genetic predictors for depression and so an alternate strategy of in-depth phenotyping should be pursued in tandem.

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ORIGINAL ARTICLE

Estimating the heritability of reporting stressful life events captured by common genetic variants

R. A. Power[1]*, T. Wingenbach[1], S. Cohen-Woods[1], R. Uher[1,2], M. Y. Ng[1], A. W. Butler[1,3], M. Ising[4], N. Craddock[5], M. J. Owen[5], A. Korszun[6], L. Jones[7], I. Jones[6], M. Gill[8], J. P. Rice[9], W. Maier[10], A. Zobel[10], O. Mors[11], A. Placentino[12], M. Rietschel[13], S. Lucae[4], F. Holsboer[4], E. B. Binder[4], R. Keers[1,6], F. Tozzi[14], P. Muglia[14,15,16], G. Breen[1,17], I. W. Craig[1], B. Müller-Myhsok[4], J. L. Kennedy[18,19], J. Strauss[18,19], J. B. Vincent[18,19], C. M. Lewis[1], A. E. Farmer[1] and P. McGuffin[1]

[1] *MRC Social Genetic and Developmental Psychiatry Centre, Institute of Psychiatry, King's College London, UK*; [2] *Department of Psychiatry, Dalhousie University, Halifax, NS, Canada*; [3] *Department of Psychiatry, University of Hong Kong, Hong Kong, Special Administrative Region, China*; [4] *Max Planck Institute of Psychiatry, Munich, Germany*; [5] *MRC Centre for Neuropsychiatric Genetics and Genomics, Neuroscience and Mental Health Research Institute, Cardiff University, UK*; [6] *Barts and The London Medical School, Queen Mary University of London, UK*; [7] *Department of Psychiatry, School of Clinical and Experimental Medicine, University of Birmingham, UK*; [8] *Department of Psychiatry, Trinity Centre for Health Science, Dublin, Ireland*; [9] *Department of Psychiatry, Washington University, St Louis, MO, USA*; [10] *Department of Psychiatry, University of Bonn, Germany*; [11] *Centre for Psychiatric Research, Aarhus University Hospital, Risskov, Denmark*; [12] *Psychiatric Unit 23, Department of Mental Health, Spedali Civili Hospital and Biological Psychiatry Unit, Centro San Giovanni di Dio, FBF, Brescia, Italy*; [13] *Division of Genetic Epidemiology in Psychiatry, Central Institute of Mental Health, Mannheim, Germany*; [14] *GlaxoSmithKline Research and Development, Verona, Italy*; [15] *Department of Psychiatry, University of Toronto, Canada*; [16] *NeuroSearch A/S, Ballerup, Denmark*; [17] *NIHR Biomedical Research Centre for Mental Health, South London and Maudsley NHS Foundation Trust and Institute of Psychiatry, King's College London, UK*; [18] *Neurogenetics Section, Centre for Addiction and Mental Health, Toronto, Canada*; [19] *Department of Psychiatry, University of Toronto, Canada*

Background. Although usually thought of as external environmental stressors, a significant heritable component has been reported for measures of stressful life events (SLEs) in twin studies.

Method. We examined the variance in SLEs captured by common genetic variants from a genome-wide association study (GWAS) of 2578 individuals. Genome-wide complex trait analysis (GCTA) was used to estimate the phenotypic variance tagged by single nucleotide polymorphisms (SNPs). We also performed a GWAS on the number of SLEs, and looked at correlations between siblings.

Results. A significant proportion of variance in SLEs was captured by SNPs (30%, $p=0.04$). When events were divided into those considered to be dependent or independent, an equal amount of variance was explained for both. This 'heritability' was in part confounded by personality measures of neuroticism and psychoticism. A GWAS for the total number of SLEs revealed one SNP that reached genome-wide significance ($p=4\times10^{-8}$), although this association was not replicated in separate samples. Using available sibling data for 744 individuals, we also found a significant positive correlation of $R^2=0.08$ in SLEs ($p=0.03$).

Conclusions. These results provide independent validation from molecular data for the heritability of reporting environmental measures, and show that this heritability is in part due to both common variants and the confounding effect of personality.

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Key words: GCTA, heritability of environment, personality, stressful life events.

Introduction

As a better understanding of the complex interplay between genes and environment has emerged, some of the simpler notions of 'nature *versus* nurture' have fallen out of favour. Nowhere is this more obvious than in studies showing that many environmental measures in fact show substantial heritability (Plomin *et al*. 1990; Kendler & Baker, 2007). One such environmental factor is the adversity, or stressful life events (SLEs), an individual has encountered. SLEs have been known to play an important role in the development of mental disorders, for example as a trigger for the onset of major depression (Kendler *et al*. 1999*b*), psychosis (Bebbington *et al*. 1993; Kessing *et al*. 2004) and

* Address for correspondence: Mr R. A. Power, MRC Social, Genetic and Developmental Psychiatry Centre, Institute of Psychiatry, London SE5 8AF, UK.
(Email: robert.r.power@kcl.ac.uk)

mania (Kennedy *et al*. 1983). Although SLEs might seem to be purely external environmental factors, there is evidence from twin studies for a genetic component ranging from 20% to 50% (Plomin *et al*. 1990; Kendler *et al*. 1993; Billig *et al*. 1996) when self-report measures are used. Longitudinal studies have also shown that patterns in reporting SLEs are consistent for individuals through life (Andrews, 1981; Woods *et al*. 1982). This heritability is often only found for SLEs that are considered 'dependent', that is potentially influenced by an individual's behaviour, suggesting that heritable aspects of an individual's behaviour may play a role in their choice of environment and exposure to SLEs, with 'independent' events often found to lack any heritable component (Kendler *et al*. 1999*a*; Bemmels *et al*. 2008; Boardman *et al*. 2011). Furthermore, there is some evidence that this heritability is, at least in part, mediated by factors such as personality (Saudino *et al*. 1997; Kandler *et al*. 2012). This may reflect gene–environment correlation, where genetically controlled behaviours make an individual more likely to encounter or seek out stressful environments (e.g. a propensity towards risk taking). Alternatively, this could reflect heritable personality types more likely to interpret or report events as stressful. This is supported by studies showing increased heritability of self-reported events compared to objective measures (Thapar & McGuffin, 1996; Kendler & Baker, 2007).

Although quantitative genetic investigations have shown heritability of SLEs, no molecular research has been carried out on known environmental measures to date. To expand upon the results of twin studies, we first aimed to look at the correlation between siblings in the reporting of SLEs to establish familiarity. Second, we estimated the additive genetic variance explained by single nucleotide polymorphisms (SNPs) for the reporting of SLEs, using the software tool genome-wide trait association analysis (GCTA; Yang *et al*. 2011). We also examined if these estimates of the heritability captured by common SNPs varied with regard to those events supposedly dependent or independent of an individual's actions, or after correcting for personality measures. Third, we sought to identify specific genetic variants associated with the reporting of SLEs in a genome-wide association study (GWAS).

Method

Samples

The sample consists of individuals from the Depression Case Control (DeCC) study (Cohen-Woods *et al*. 2009) and the Depression Network (DeNT) study (Farmer *et al*. 2004). Both were retrospective studies of DSM-IV/ICD-10-defined recurrent depression of at least moderate severity. The controls were recruited through the Medical Research Council (MRC) general practice research framework, and screened for any history of psychiatric disease. DeCC provided 2327 individuals (1222 cases and 1106 controls) from the UK with both phenotype and genotype data. DeNT provided 744 sibling pairs with phenotype data for correlation analysis, and 294 genotyped individuals for genetic analysis. Only UK individuals were used for genetic analyses to avoid population stratification. The studies were approved by the local ethical committees and informed written consent was obtained from all participants.

Measures

The SLE data were derived from the Brief Life Event Questionnaire (BLEQ), which consisted of 12 items and was based on the List of Threatening Experiences Questionnaire (LTE-Q; Brugha *et al*. 1985). Individuals had to state whether or not they experienced the respective SLEs within the past 6 months. As this was a retrospective study, cases were not selected for in episode. Response categories were defined in a binary fashion, either present or absent, and a sum of the number of reported SLEs was constructed for every individual. Following the LTE-Q categories, SLEs were split into those considered dependent on an individual's actions and those that were deemed to be independent. Illness, death and being robbed were considered as independent SLEs; unemployment, separation, financial problems and legal matters as SLEs dependent on individuals' behaviour. This totalled five independent events and seven dependent events. These sums of numbers of independent and dependent events were then used in secondary analyses.

Personality was evaluated using the revised Eysenck Personality Questionnaire (EPQ-R), with separate measures for extraversion, psychoticism and neuroticism (Eysenck & Eysenck, 1975; Eysenck *et al*. 1985). Each of these personality measures was used individually as a covariate to test for confounding in the heritability of reporting SLEs.

Genotyping and quality control

For the whole sample Illumina Human Hap610-Quad BeadChips [Centre National de Genotypage (CNG), France] were used for genotyping. The genotyping is described in more detail elsewhere (Lewis *et al*. 2010). Individuals were excluded if the missing rate of genotypic data was >1%, the data showed abnormal

heterozygosity or discrepant gender assignment, or the participants were close relatives (up to second degree) or of non-European ancestry. If SNPs showed minor allele frequency $<1\%$, missingness of $>1\%$ or departure from Hardy–Weinberg equilibrium ($p<1\times10^{-5}$), they were excluded.

Statistical analyses

Sibling correlation for SLEs

From the DeNT sample one sibling pair was selected from each family. Spearman's correlation was conducted in Stata (StataCorp, 2011) for all SLEs, and for independent and dependent SLEs separately. For this analysis we also created a sum of SLEs that were not defined by familial events; thus the items 'death of parent', 'serious illness, injury or assault of a close relative' and 'death of close family friend or another relative' were excluded.

GCTA

GCTA was implemented to investigate the phenotypic variance in the number of SLEs explained by all SNPs (Yang *et al*. 2011). Analyses were conducted for all SLEs and separately for those considered dependent or independent. Correction for case–control status, study, genotyping batch and the first five principal components was applied. The addition of further principal components did not seem to alter the findings. Individuals were also screened for relatedness, and in any pair where $R>0.025$, one individual was excluded. To test the effect of personality, we then repeated this analysis correcting for psychoticism, neuroticism and extraversion separately.

GWAS

To identify specific genetic variants related to the number of reported SLEs, GWAS were conducted for all SLEs, and for dependent and independent SLEs separately, using PLINK (Purcell *et al*. 2007). The threshold for genome-wide significance was set to $p<5\times10^{-8}$, and $p<5\times10^{-6}$ was used for suggestive significance (Dudbridge & Gusnanto, 2008). The first two ancestry informative principal components were included as covariates and no inflation of genomic control values above $\lambda=1.05$ was seen.

Replication

Top hits from the GWAS were replicated within two separate samples. The first was a cohort of controls from a bipolar dataset from Toronto, collected as part of the bipolar disorder arm of the RADIANT studies, to which DeCC and DeNT belong (Gaysina *et al*. 2009). They totalled 257 individuals with phenotype and genotype information, collected in a similar manner to the DeCC and DeNT studies. The second was a set of 894 cases from a clinical depression cohort of individuals in Munich, again collected in a similar manner to the DeCC and DeNT studies (Tozzi *et al*. 2008; Muglia *et al*. 2010). For the Munich sample, individual event data were not available, only a summary score for the BLEQ for cases. Therefore, this sample could be used for replicating findings for the total number of events, but not hits specific to dependent or independent events.

Results

Sibling correlation for SLEs

Altogether, 744 sibling pairs were available to test the familiality of SLEs. Spearman's correlation was conducted to investigate the relationship between the number of SLEs reported by siblings. Significant positive correlations were found for all variables, with the total number of events correlating at 0.19 ($p<0.001$). Both independent and dependent events were correlated, although independent events to a much greater degree (an R^2 of 0.25 compared to 0.08, with $p<0.001$ and $p=0.03$ respectively). This is probably because independent events included many that were related to illness or death of family members and an analysis of total events, excluding those specifically family related, produced results similar to just dependent events ($R^2=0.08$, $p=0.03$).

GCTA

After quality control, genome-wide association data were available from 2578 unrelated individuals and 541628 SNPs. The composition of these samples is outlined in Table 1, with the average number of events in the past 6 months prior to interview per person near to one in all. The correlation between total number of independent and dependent events was 0.15. The results from the GCTA show that a significant proportion of variance could be attributed to common genetic variants for all, dependent and independent SLEs. The phenotypic variance accounted for by all SNPs for the number of reported SLEs was 29% ($p=0.03$, S.E. $=0.16$). When distinguishing between SLEs dependent on and independent of subjects' behaviour, SNPs explained 30% of the variance ($p=0.03$, S.E. $=0.16$) for dependent events and 26% ($p=0.04$, S.E. $=0.15$) for independent events. The GCTA was rerun after correcting for the effects of personality, giving the results outlined in Table 2. The total variance explained by SNPs for the number of SLEs was

Table 1. *Description of studies and the distribution of stressful life events (SLEs)*

Study	Genotyped UK individuals	Cases (%)	Female (%)	Mean age (years)	Mean number of SLEs (S.E.)
DeCC	2327	52.5	64.7	44.3	1.0 (0.26)
DeNT	294	100	76.2	45.6	1.3 (0.09)
Toronto	257	0	52.1	41.8	1.1 (0.08)
Munich	864	100	67.3	50.8	2.3 (0.11)[a]

DeCC, Depression Case Control study; DeNT, Depression Network study; S.E., standard error.

[a] Note that for the Munich replication sample, the mean Brief Life Event Questionnaire (BLEQ) score is displayed rather than the number of events.

no longer found to be significant after correcting for either neuroticism (from 29% to 23%, $p=0.07$) or psychoticism (to 17%, $p=0.15$). Neuroticism scores did not affect GCTA estimates when events were separated into those considered dependent on or independent of an individual's actions, and psychoticism only confounded the analysis of dependent events. Accounting for extraversion scores did not affect the results.

GWAS

For the analysis of the number of SLEs as a continuous trait in 2578 cases, the study had 50% power to detect association with an SNP with a frequency of 25%, accounting for 1.1% variation in SLEs, or 80% power for 1.5% variation. No genome-wide significant results were found for the total number of events in the genome-wide association, although five SNPs reached suggestive significance. One of these (rs4927134 on chromosome 1) reached genome-wide significance when only dependent events were included. One SNP not found in the primary analysis of all events (rs16837293) reached suggestive significance when only dependent events were included, and one of the SNPs in the primary analysis (rs17040523) was found to be in suggestive significance when using only independent events. Details of the SNPs with the strongest association found are given in Table 3. None of these top hits reached even nominal significance in the replication samples ($p<0.05$).

Discussion

This study has provided new molecular evidence showing that common genetic variants explain a significant proportion of the variance in self-reported environmental factors. Twin studies have previously suggested a heritability of approximately 20–50% for the number of reported SLEs (Plomin *et al*. 1990; Kendler *et al*. 1993; Billig *et al*. 1996) and, in keeping with this, we found that ~30% of the phenotypic variance in SLEs was tagged by common SNPs. A degree of underestimation is expected in this study as only common single nucleotide variants were genotyped, often relying on linkage disequilibrium to tag the true number of common variants, and missing copy number or rare variants entirely. This may suggest that the 'true' heritability is towards the top end of the range found in twin studies. Of note, there was no clear difference in the variance explained for supposedly independent or dependent SLEs, although the confidence intervals are large. This is in contrast to the findings from twin studies, but may reflect the fairly crude division of independent and dependent events in this analysis.

Regarding the role of personality, correcting for either neuroticism or psychoticism resulted in a non-significant heritability of the total number of SLEs, with psychoticism also showing a confounding effect when analysis was restricted to dependent events. Psychoticism has previously been associated with increased reckless and impulsive behaviour (Pickering *et al*. 2003), which may impact the risk of those events likely to be labelled 'dependent' to a greater extent. Neuroticism has been associated with a high level of depression symptoms (Farmer *et al*. 2002), and perhaps its effect captured a difference in the sample due to inclusion of depressed individuals that was not fully corrected for. This highlights one of the main limitations of this study, which is the inclusion of cases and controls. Despite correcting for affected status and using the number of events at 6 months before interview rather than before episode, the results may not be entirely reflective of a sample from the general population. Unfortunately, because of the need for large samples, the heritability in controls alone could not be tested. These results are, however, in line with findings from twin and family studies, showing that neuroticism accounts for a portion of the heritability

Table 2. *Variance explained by common SNPs for total stressful life events (SLEs), and split into those considered dependent or independent of an individual's state of mind. Also shown are the adjusted estimates after correcting for the personality measures neuroticism, extraversion and psychoticism separately*

				Neuroticism		Extraversion		Psychoticism	
Events	Heritability	S.E.	*p* value	h^2	*p* value	h^2	*p* value	h^2	*p* value
All events	0.29	0.16	0.03	0.23	0.07	0.28	0.04	0.17	0.15
Dependent	0.30	0.16	0.03	0.29	0.04	0.31	0.03	0.20	0.11
Independent	0.26	0.15	0.04	0.27	0.04	0.28	0.04	0.27	0.04

SNP, Single nucleotide polymorphism; SLE, stressful life event; S.E., standard error.

Table 3. *SNPs with the strongest signals for associations in GWAS of the number of reported SLEs, and their associations in the two replication samples: controls from a bipolar sample in Toronto and cases from a depression study in Munich (which lacked information on whether the SLEs were independent or dependent)*

					RADIANT ($n=2578$)		Toronto ($n=257$)		Munich ($n=894$)		
Events	CHR	SNP	BP	Risk allele	β	*P*	β	*p*	β	*p*	Sign
All	1	rs4927134	54 756 695	C	0.28	5.8×10^{-8}	0.20	0.25	0.13	0.58	+ + +
All	7	rs758938	31 765 832	A	−0.16	1.2×10^{-6}	−0.12	0.31	0.14	0.33	− − +
All	2	rs17040523	2 874 200	T	0.26	1.3×10^{-6}	0.22	0.22	0.05	0.83	+ + +
All	7	rs11980485	31 779 361	G	−0.16	1.4×10^{-6}	−0.12	0.30	0.13	0.37	− − +
All	1	rs10494809	198 398 707	T	0.18	3.0×10^{-6}	−0.14	0.27	0.35	0.05	+ − +
Dependent	**1**	**rs4927134**	**54 756 695**	**C**	**0.20**	$\mathbf{4.1\times10^{-8}}$	**0.09**	**0.50**	–	–	+ +
Dependent	3	rs16837293	127 418 611	G	0.27	2.9×10^{-6}	−0.28	0.19	–	–	+ −
Independent	2	rs17040523	2 874 200	T	0.16	5.5×10^{-7}	0.11	0.24	–	–	+ +

SNP, Single nucleotide polymorphism; GWAS, genome-wide association study; SLE, stressful life event; CHR, chromosome; BP, base position; Sign, direction of effect per respective study.

of reporting stressful events (Dudbridge & Gusnanto, 2008; Kandler *et al*. 2012).

The association analysis of specific SNPs provided no replicable findings, but this is not entirely surprising because of the small effect sizes reported in associations with even the clearest of phenotypes. None of the SNPs found at suggestive significance were associated with personality disorders or other candidate genes. A strong positive correlation for the number of reported SLEs among siblings was also found, with the highest correlation for independent SLEs. This finding is self-explanatory as life events were included that overlap because of relatedness, for example 'serious illness of close relative', although excluding such items still leads to a significant positive correlation. This could be evidence for the role of genetic similarity, but of course could be due to shared environmental factors.

Taking together the results from the GWAS, GCTA and sibling comparison, we add to the now considerable evidence that the reporting of SLEs is heritable. Our results also support studies showing that at least part of this heritability can be attributed to confounding from heritable personality measures. It should be noted, however, that in this study we relied entirely on self-reporting, and using such 'soft' measures meant we were unable to differentiate between the heritability of propensity towards reporting SLEs and the heritability of experiencing SLEs. Nonetheless, this finding is of relevance to studies exploring gene–environment interactions, where gene–environment correlation may be a concern. It also provides more insight into the complexities of disentangling the heritable component of a disorder, such as depression, that is often associated with stressful events.

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Declaration of Interest

K. J. Aitchison, A. E. Farmer and P. McGuffin have received consultancy fees and honoraria for participating in expert panels for pharmaceutical companies including GlaxoSmithKline. K. J. Aitchison declares interests through Advisory Boards for Johnson & Johnson, Lundbeck, Roche Diagnostics and Bristol–MyersSquibb; membership of the Bristol–Myers–Squibb UK Steering Group, 2003 to present; consultancy work for Roche Diagnostics, Johnson & Johnson Pharmaceutical Research and Development, Lundbeck, and Bristol–MyersSquibb Pharmaceuticals Limited; grants awarded by Johnson & Johnson Pharmaceutical Research and Development, Bristol-Myers Squibb Pharmaceuticals Limited, and E. Merck Pharmaceuticals. F. Tozzi and P. Muglia were employees of GlaxoSmithKline when the research was performed. M. Ising has received consultancy honoraria from MSD Merck. E. B. Binder has received grant support from PharmaNeuroboost.

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ORIGINAL ARTICLE

Genetic predisposition to schizophrenia associated with increased use of cannabis

RA Power[1,2], KJH Verweij[3], M Zuhair[1], GW Montgomery[4], AK Henders[4], AC Heath[5], PAF Madden[5], SE Medland[4], NR Wray[2] and NG Martin[4]

Cannabis is the most commonly used illicit drug worldwide. With debate surrounding the legalization and control of use, investigating its health risks has become a pressing area of research. One established association is that between cannabis use and schizophrenia, a debilitating psychiatric disorder affecting ~1% of the population over their lifetime. Although considerable evidence implicates cannabis use as a component cause of schizophrenia, it remains unclear whether this is entirely due to cannabis directly raising risk of psychosis, or whether the same genes that increases psychosis risk may also increase risk of cannabis use. In a sample of 2082 healthy individuals, we show an association between an individual's burden of schizophrenia risk alleles and use of cannabis. This was significant both for comparing those who have ever versus never used cannabis ($P = 2.6 \times 10^{-4}$), and for quantity of use within users ($P = 3.0 \times 10^{-3}$). Although directly predicting only a small amount of the variance in cannabis use, these findings suggest that part of the association between schizophrenia and cannabis is due to a shared genetic aetiology. This form of gene–environment correlation is an important consideration when calculating the impact of environmental risk factors, including cannabis use.

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INTRODUCTION

During the last quarter of the 20th century, cannabis use has increased to become the most widely used illicit drug in the world.[1] It is well established that cannabis use is much higher among schizophrenic patients than in the general population.[2] Cannabis intoxication can lead to an acute transient psychotic episode and produce short-term exacerbations of pre-existing psychotic symptoms,[3–5] an association that has been confirmed through the experimental administration of tetrahydrocannabinol.[6,7] Meta-analyses of prospective studies have found that cannabis use increases the likelihood of developing a psychotic illness by a factor of roughly two.[8–11] A dose response effect has been demonstrated,[12–14] and use in adolescence has been associated with the greatest risk.[15] Given the large health burden from schizophrenia and other psychotic disorders,[16] the view that cannabis use is a component cause of schizophrenia has heavily influenced discussion over the legislation surrounding cannabis use.

However, the relationship between schizophrenia and cannabis use may be more complicated than it initially seems. Despite a clear association between the two, the possibility of reverse causation has not been entirely excluded. Some small studies have suggested that it is in fact psychosis that is a risk factor for cannabis use, as those on a psychotic spectrum are more likely to experiment with drugs.[17,18] The strongest evidence comes from Ferdinand *et al.*[19] who found that the association was bidirectional, as cannabis-naive children with prodromal psychotic episodes had greater incidence of later cannabis use. However, a similarly sized study failed to replicate this finding.[20] There is also the possibility of attempts by patients at self-medication, as it has been suggested that cannabis use can reduce negative and affective symptoms in patients with an established psychotic disorder.[21–23]

The issue is further complicated by tentative evidence for interactions between cannabis use and genetic risk variants for schizophrenia.[24] Schizophrenia is known to be highly heritable with up to 80% of the variance explained by additive genetic effects,[25] and as sample sizes have increased a growing number of genetic risk variants have been identified.[26,27] Interactions between risk variants and cannabis use might explain why some individuals experience psychosis while others do not. However, cannabis use itself has been reported to be heritable,[28–30] although no genetic risk variants have been identified.[31] It is unclear to what extent the heritability of cannabis use results from shared heritability with other behavioural phenotypes such as schizophrenia predicting its use.

Here we test for such genetic overlap directly, and aim to discern the direction of causation between cannabis use and schizophrenia. Within a sample of 2082 healthy individuals, we tested to see whether polygenic risk scores for schizophrenia predict cannabis use. Polygenic risk scores reflect the cumulative burden of risk alleles carried by an individual as identified in a previous genome-wide association study (GWAS),[32] here of 13 833 schizophrenia cases and 18 310 controls.[27] Such an association with cannabis use would suggest that those genetically predisposed to schizophrenia use cannabis more frequently. This would mean that the association between schizophrenia and cannabis use is not simply one of an environmental risk factor, but rather involves gene–environment correlation, as individuals

[1]MRC Social, Genetic & Developmental Psychiatry Centre, Institute of Psychiatry, King's College London, London, UK; [2]Queensland Brain Institute, The University of Queensland, St Lucia, QLD, Australia; [3]Department of Developmental Psychology and EMGO Institute for Health and Care Research, VU University, Amsterdam, The Netherlands; [4]QIMR Berghofer Medical Research Institute, Brisbane, QLD, Australia and [5]Department of Psychiatry, Washington University School of Medicine, St Louis, MO, USA. Correspondence: R Power, MRC Social, Genetic & Developmental Psychiatry Centre, Institute of Psychiatry, King's College London, DeCrespigny Park, Denmark Hill, London SE5 8AF, UK.
E-mail: robert.r.power@kcl.ac.uk
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choose and shape their own environment based on their own innate preferences.

MATERIALS AND METHODS

The data used in this study come from the Australian Twin Registry. Data were obtained from two studies in which twins and their families participated in semi-structured diagnostic telephone interviews aimed primarily at assessing psychiatric health. Informed consent was obtained from all participants.

Sample 1 consisted of 6265 individuals aged between 23 and 39 years (mean = 29.9 ± 2.5) interviewed between 1996 and 2000. Participants were members of the young adult cohort, a volunteer panel of twins born between 1964 and 1971. The interview was based on a modified version of the SSAGA (Semi-Structured Assessment of the Genetics of Alcoholism[33]). Detailed information about the sample recruitment, the study procedure and the measures can be found elsewhere.[34] Sample 2 comprised 9688 individuals aged between 18 and 91 years (mean = 46.3 ± 11.3) interviewed between 2001 and 2005. Participants were members of the older and younger adult cohort of Australian twin pairs (born between 1895 and 1964, and between 1964 and 1971, respectively). A subset of this sample was ascertained based on large sibship size, or having a relative with nicotine or alcohol dependence. The interview used for this sample was also based on a modified version of the SSAGA. Further details about the sample and assessment can be found in Heath *et al*.[35]

A subset of the participants ($N = 1866$; 11.7%) participated in both studies, in which case we used data from the last assessment. The combined phenotypic sample consisted of 14 087 individuals, of whom 7172 were genotyped. In both studies, twins were asked the same items about cannabis use: (1) did you ever use marijuana?, (2) how old were you the very first time you tried marijuana (not counting the times you took it as prescribed)? and (3) how many times in your life have you used marijuana (do not count times when you used a drug prescribed for you and took the prescribed dose). Ever use was measured on a dichotomous scale (ever versus never), whereas age at initiation and quantity of use were open questions. Table 1 shows the prevalence of cannabis use for individuals included in the present study.

Genotype data were obtained using three different Illumina single nucleotide polygmorphism (SNP) genotyping platforms (317K, HumanCNV370-Quadv3, Human CNV370v1 and Human610-Quad). Standard quality control procedures were applied as outlined previously,[36] including checks for ancestry outliers, Hardy–Weinberg equilibrium ($P < 10^{-6}$), Mendelian errors, call rate, genotypic missingness (>5%), individual missingness (>5%) and minor allele frequency (< 0.01). Individuals were pruned on relatedness, removing one individual from each pair with relatedness >0.05, as determined from genetic data. The final sample therefore comprised 2082 'unrelated' individuals (see Table 1 for sample details).

Polygenic risk scores were constructed using the *P*-values and $\log_{10}$ odds ratios from the most recent large GWAS of schizophrenia, a meta-analysis of the Psychiatric Genomics Consortium's studies with additional Swedish samples totalling 13 833 cases and 18 310 controls.[27] SNPs were pruned for linkage disequilibrium using *P*-value informed clumping in PLINK,[37] using a cutoff of $R^2 = 0.25$ within 200 kb window. The major histocompatibility complex region of the genome was excluded, due to its complex linkage disequilibrium structure. After linkage disequilibrium pruning, 147 830 SNPs remained. Multiple scores were generated for each individual using the PLINK score option and based on top SNPs from the schizophrenia GWAS using varying significance thresholds ($P = 0.0001$, 0.001, 0.01. 0.05, 0.1, 0.2, 0.3, 0.4, 0.5 and 1.0). Polygenic risk scores were tested for association with a binary ever versus never used cannabis and two quantitative traits for quantity of use and age at first use, in logistic and linear regressions, respectively. These analyses were corrected for the first 10 ancestry-informative principal components, genotyping platform, sex, age, age squared and sex by age. Analysis was performed in STATA.[38]

Table 1. Summary statistics of sample for cannabis use traits

	Users	*Non-users*
N	1011	1071
Mean age (s.e.)	41.3 (0.23)	53.0 (0.37)
Percentage female (%)	46.5	56.0
Mean age at initiation (s.e.)	19.6 (0.06)	—
Mean number of uses over lifetime (s.e.)	62.7 (4.56)	—

RESULTS

After pruning, 2082 unrelated individuals remained in our sample with both genotype and phenotype measures. Within the sample, 1011 individuals (48.6%) had ever used cannabis, of whom 997 had data on quantity of use. Mean number of usages of cannabis over lifetime was 62.7 (95% CI 53.8–71.6), and the mean age of initiation of use was 20.1 (95% CI 19.7–20.5). Males showed higher rates of use than females, 53.5% compared with 43.9% ($P < 0.001$), although no significant difference in age at initiation. Table 1 shows the summary statistics for the sample.

Polygenic risk scores for schizophrenia showed positive associations for ever versus never use of cannabis across all *P*-value thresholds, with the strongest association for those SNPs with *P*-values of 0.01 or below in the original schizophrenia GWAS (see Figure 1, $R^2 = 0.47\%$, $P = 2.6 \times 10^{-4}$). Significant associations were also seen in the analysis of quantity of cannabis use for 9 of the 10 SNP cutoffs, with the top association seen for those SNPs with $P \leqslant 0.05$ for schizophrenia ($R^2 = 0.85\%$, $P = 0.003$). No association was seen with age at initiation of use, although the association with quantity of use remained significant when number of years of usage was accounted for (results not shown).

As a secondary analysis, polygenic risk score for schizophrenia risk alleles with $P \leqslant 0.01$ (the threshold with the greatest association in the primary analysis) was examined within 990 twin pairs (608 dizygotic and 382 monozygotic) where data on cannabis use of both twins was available. Taking the mean polygenic risk score within each twin pair, an ordinal regression was performed to predict whether neither ($n = 272$), one ($n = 273$) or both twins ($n = 445$) were cannabis users. After correcting for age, sex and zygosity, a significant association was observed ($P = 0.001$). Those twin pairs where both reported using cannabis had the greatest burden of schizophrenia risk alleles, pairs with only one user were found to have an intermediate level and the

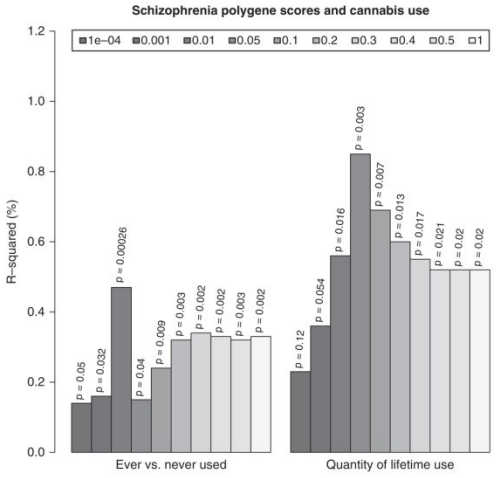

Figure 1. Results of polygenic risk scores for schizophrenia predicting variance explained (R^2) in cannabis use as both a binary trait of ever versus never, and as a quantitative trait of lifetime use within only users. Polygenic scores were created using different cutoffs for the inclusion of risk variants for schizophrenia, ranging from $P = 0.0001$ to 1.0.

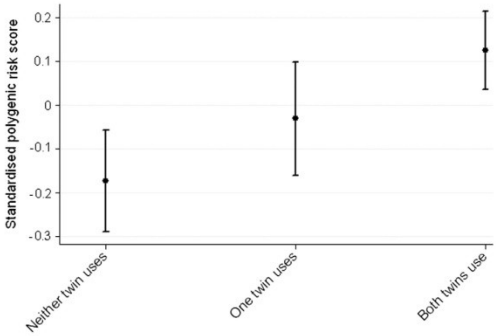

Figure 2. Mean standardized schizophrenia polygenic risk scores for pairs of twins when neither ($n = 272$), one ($n = 273$) or both twins ($n = 445$) had reported use of cannabis. An ordinal regression reported a significant association ($P = 0.001$).

lowest burden was found in pairs where neither twin reported use (see Figure 2).

DISCUSSION

Our results show that to some extent the association between cannabis and schizophrenia is due to a shared genetic aetiology across common variants. They suggest that individuals with an increased genetic predisposition to schizophrenia are both more likely to use cannabis and to use it in greater quantities. This is not to say that there is no causal relationship between use of cannabis and risk of schizophrenia, but it does establish that at least part of the association may be due to causal relationship in the opposite direction. Although the variance in cannabis use explained by schizophrenia polygenic risk scores is small, it is in line with other cross-phenotype analyses, largely due to the polygenic risk scores for schizophrenia predicting only ~7% of the variation for schizophrenia itself. Previous associations between polygenic risk scores for schizophrenia and other psychiatric illnesses, such as bipolar disorder, major depression and autism,[39] have shown effects of similar sizes. Further research will be needed to see whether the genetic overlap observed here is specific to cannabis use or is present across illicit drug use and addiction phenotypes, data for which was not widely available in this sample. For now, these findings have important implications for the current perception of cannabis use as a risk factor for schizophrenia, and other psychotic disorders.

However, it is worth noting that this association, if true, does not rule out the possibility of cannabis independently being a risk factor for schizophrenia. A bidirectional association between cannabis use and psychosis has previously been suggested.[40] Further, one caveat to interpreting the direction of causation concerns the discovery sample used to identify schizophrenia risk alleles. The schizophrenia GWAS sample will likely include many more cannabis users among cases than controls. This may lead to an excess of causal SNPs associated with cannabis use, as opposed to schizophrenia itself, identified as schizophrenia risk alleles. Only if the discovery schizophrenia sample was comprised entirely of non-cannabis users could causation be inferred without any risk of confounding. This is an important consideration as to whether polygenic risk scores overestimate individuals' un-modifiable genetic risk by including their genetic predisposition to modifiable environmental risk factors.

These results highlight the blurring between behavioural phenotypes and environment, and have wider implications for how we perceive supposedly environmental risks for disease. Individuals select their own environments based on their innate and learned preferences, and have their environments react to their own behaviour. Further, parents pass down both genes and environment to their children. All of these can contribute to gene–environment correlation, particularly with respect to behavioural traits. Several studies have shown that supposedly environmental risk factors such as urbanicity, religiosity and stressful life events have heritable components to them.[41–43] The existence of heritability for supposedly environmental risk factors does not mean they are inevitable, only that causality is more complicated to discern. Future studies will need to explore the matching of cases and controls on environmental risk variants to fully disentangle causation. This can be supplemented exploring the generation of polygenic risk scores for environmental risk factors, and their role in predicting disease status. The wider availability of genetic data in richly phenotyped samples should allow for the integration of genetics into an epidemiological framework, and so the discovery of gene–environment correlations where they exist.

With ongoing debate over the legalization of cannabis and the potential health risks it poses, understanding the association between its use and schizophrenia is a priority. It has previously been suggested that, even assuming an entirely causal relationship, the required reduction in the number of cannabis users to prevent one case of schizophrenia is in the thousands.[44] Our findings here highlight the possibility that this association might be bidirectional in causation, and that the risks of cannabis use could be overestimated. This is an important subtlety to consider when calculating the economic and health impact of cannabis use.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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8. Discussion

8.1 Summary

Psychiatric disorders reflect a wide range of illnesses that, while showing substantial aetiological and diagnostic overlap, vary greatly in prevalence, symptoms, onset, and heritability. The work presented in this thesis began by looking at the impact of these disorders on reproductive fitness, to understand if they are likely to undergo the same selection pressures and so have similar underlying genetic architectures. This identified that depression was not under negative selection, a finding that was then explored through molecular data. These results suggested the potential role of gene-environment interactions as an adaptive mechanism for variants contributing risk to depression, and were followed up by studies focusing on identifying such interactions. The conclusion of the thesis was though no gene-environmental interaction could be found to explain how the risk variants for major depression avoided negative selection, this may be due to substantial gene-environment correlation in the reporting and experiencing of environmental risk factors in psychiatric disorders which would confound such analyses. A more detailed discussion follows of the findings and how they relate to each other.

8.2 Overview of Findings

The first paper of the thesis examined epidemiological data from Sweden for evidence of the selection on psychiatric traits [Power and others 2013b]. The most striking finding of this analysis was that affected males showed a much greater reduction in fecundity than affected females, suggesting that psychiatric illness impacts the fitness of men to a greater extent and there may be sex-specific selection on the causal genetic variants. On an individual disorder basis, autism and schizophrenia showed the expected large reduction in fecundity within patients suggesting that genetic variants that predispose to risk are under negative selection and are maintained by *de novo* mutations. Anorexia, substance abuse and bipolar disorder showed a moderate reduction in fecundity, suggesting the role of *de novo mutations* may be less important. This supports a

distinction between bipolar disorder and schizophrenia in the literature of molecular studies of these disorders [Sullivan and others 2012], with a greater role of novel mutations observed in schizophrenia. Surprisingly, only a very small reduction was seen in depression patients which was further restricted to affected males not females. Even more surprisingly this reduction was more than compensated by the observed increase in fecundity in the siblings of affected individuals, suggesting that having certain combinations of the risk variants and environments for depression could be beneficial to an individual's fitness. This finding was based on modern levels of fecundity which may be confounded by a number of factors, such as the effects of hospitalisation and treatment, modern stigma of the disorders, and the use of contraceptives, which may give estimates of selection on these disorders that do not relate to historical levels throughout human evolution. This is an important distinction as the genetic architecture of traits is defined by selection over previous generations, rather than selection in the current generation. In the second paper of this thesis, it was supported by findings from a molecular study of 9 studies of major depression looking for an association with runs of homozygosity (ROH) [Power and others 2014a],which can be used to infer the impact of selection on a trait over previous generations. Inbreeding, as captured by ROH, exposes recessive variants, and an excess of recessive variants in association with a trait suggests that it has previously experienced negative selection. This is due to the fact that selection rapidly removes dominant deleterious mutations but both cannot as easily act on recessive variants and acts to select dominant advantageous variants. ROH were not associated with major depression status across a meta-analysis of nine studies, suggesting overall there was no evidence for negative selection on depression over previous generations.

However individual studies within this meta-analysis showed significant associations with ROH and significant heterogeneity across studies was observed, seemingly driven in part by the country the study took place in. This result and the findings from the epidemiological study, as well as previous research from others [Caspi and others 2003], suggested the potential role of differing

environmental backgrounds determining the impact of variants predisposing to MDD on fecundity. To examine this idea further, two studies were performed within the RADIANT consortium where access to detailed environment and genotype data was available. In the third paper of this thesis the interaction between childhood and adult environmental adversity was examined [Power and others 2013c], testing the mismatch hypothesis of depression whereby individuals are primed as children for a stressful or non-stressful environment in adulthood and the disorder manifests when this expectation is not met [Nederhof and Schmidt 2012] . This hypothesis also puts forward that some individuals show plasticity and can be 'programmed' by early experiences to develop behaviours that will maximise future gain (e.g. calibrating their level of anxiety to avoid harm but not be overly conservative), while other individuals show no plasticity but are more robust to environmental stressors. In this model of depression, the risk variants for depression would not always confer risk but rather are likely 'plasticity' markers of an individual's predisposition for priming in childhood or potentially their predisposition to misjudge the level of stress they should be primed for. However no evidence for the mismatch hypothesis was found in the interaction of environmental stressors across life or with a potential plasticity genetic risk variant (5-*HTTPLR*). This may have been due to the severity of the environmental stressors used, particularly the level of childhood maltreatment which was often severe, or the very improbable likelihood of success from using a candidate gene over an established replicated variant (which are currently lacking for depression) [Ripke and others 2013b]. The fourth paper of this thesis focused on a novel approach to indentifying novel variants for major depression not by looking for their interaction with environmental stressors, but by matching cases and controls on environment [Power and others 2013a]. In this analysis we observed no such variants, likely due to the large reduction in sample size that resulted from including only cases and controls with overlapping levels of stressful life events and the large discrepancy in their distributions in the two groups. In fact we observed a noticeable deflation in the expected number of associations when cases and controls were matched

on their reported number of stressful life events, suggesting that even adding more samples was unlikely to increase our chances of identifying novel genetic variants.

This deflation of associations may have been due to overcorrecting if the genetics of depression correlates with the reporting of stressful life events, or the genetics of reporting or experiencing such events. Stressful life events are themselves are known to be heritable [Billig and others 1996; Kendler and others 1993; Plomin and others 1990], as their likelihood of occurring is to some extent the result of an individual's personality and actions. In the last two papers of thesis we explored this in two ways. The fifth paper of the thesis focused on looking at the heritability of stressful life events, or at least the heritability of reporting such events, making use of genome-wide heritability estimation methods to calculate the heritability of a trait directly captured by molecular data rather than from an adoption or twin design [Power and others 2013d]. Such methods work by using genetic data to estimate the very low levels of relatedness between each pair of 'unrelated' individuals within the sample, as estimating to what extent this relatedness explained variation in phenotype. This found substantial heritability in stressful life events captured by the common variants observed, that appeared to be driven primarily by an individual's level of neuroticism. This itself is a heritable trait and suspected to overlap with depression in aetiology and definition, suggesting that those who are predisposed to being more depressed report more stressful life events. This may reflect a difference in perception of one's environment that may precede the onset of the disorder and confound the analysis of triggers of depression, and highlights the importance of having rigorously defined and recorded environmental measures due to the possibility of unreliable reporting. The sixth and last paper of this thesis showed that this gene-environment correlation is not restricted to the reporting of stressful events but also observed for traits such as drug use, showing that healthy individuals in the general population with a greater genetic predisposition to schizophrenia as defined by polygenic scoring are more likely to use cannabis [Power and others 2014b]. Polygenic scoring is a method that takes the findings from a genome-

wide association study to define which variants are more common in cases than controls (regardless of significance) and then counting them up in each individual of a independent sample. This provides each individual with a polygenic risk score which can be used to predict the same trait or other traits to test for genetic overlap, in this case taking the results from the most recent genome-wide association study of schizophrenia [Ripke and others 2013a] to test their cumulative association with cannabis use. This showed an association with both whether an individual had ever used cannabis and within users their quantity of use, but not with age at first use which had previously also been suggested to influence risk of psychosis. Given the well established association between cannabis use and risk of schizophrenia from epidemiological work [Arseneault and others 2004; McGrath and others 2010], this gene-environment correlations are important for how we identify and define environmental risk factors for psychiatric disorders. That is not to say that every gene-environment correlation removes the risk from environment, in fact the risk could still be mediated by the environment whereby individuals are genetically predisposed to seek out environments that are deleterious. Distinguishing situations where genes predispose individuals pleiotropically to both a disease and an environment through independent pathways (and so preventing the environment does not reduce disease risk), from situations where genes predispose individuals to an environment that increases the risk of a disease (and so mediates the pathway to risk) will be crucial for understanding when environmental interventions will yield success or not. Understanding how to integrate such gene-environment correlations into the analyses of gene-environment interactions will be crucial to developing methods to better identify the latter, and understand how genes and environment come together to predispose individuals to risk of a disorder.

8.3 Future directions

There are several potential areas of research that expand upon this work. Primarily is the wider application of polygenic methods, such as polygenic scoring and genome-wide heritability

estimates such as those available from GCTA [Purcell and others 2009; Yang and others 2011], to gene-environment interaction studies. These allow researchers to side step the main problem of such studies to date, that they must rely either on unreliable candidate genes that often fail to replicate or on genome-wide association data that allows the identification of novel genetic variants but with much reduced power.

These methods can also continue to be applied to the search for gene-environment correlations as in this thesis, such as following up the findings between schizophrenia and cannabis by looking at other illicit drugs or addictions. However to do so without any risk of confounding, greater information on the environmental exposures will be needed across datasets. For instance in the analysis of schizophrenia and cannabis use, the polygenic scores for schizophrenia were based on a genome-wide association study of schizophrenia that was blind to cannabis use. In this scenario the association with cannabis use could be due to cannabis use risk variants being picked up in the schizophrenia GWAS, as cases within this study are much more likely to also be users than controls. As such the GWAS of schizophrenia, with more cases with environmental risks for schizophrenia than controls, would pick up not just genetic variants that predispose directly to schizophrenia but also any variants that predispose to environments that increase risk to schizophrenia (assuming there is a heritable component to experiencing these environments). Ideally a follow up study could be performed where the discovery GWAS of schizophrenia had cases and controls matched on cannabis use, or the GWAS restricted to non-users in both groups. This would give a more definitive understanding of how the two traits' genetics overlap. It may also give us an estimate of how much of the heritability of schizophrenia comes from genetic predisposition to deleterious environments, and so how much of the heritability is actually modifiable.

Turning away from the gene-environment section of the thesis and back to the evolutionary analyses of depression, there are implications from finding no negative selection on depression. The

effect size and frequency of 'risk' variants should be curtailed by the selection against them in the population, meaning a trait with weaker selection against it should on average have variants of larger effect and so be more easily identified. However in the case of depression this is clearly not the case, given the difficulty in robustly identifying any risk variants to date [Ripke and others 2013b]. This could in part be due to the fact that the lack of selection is due to variants being beneficial in some circumstances (as seen in the analysis of fecundity of siblings of those with depression), meaning that many seemingly healthy controls will be carriers and reducing power to detect associations.

How exactly these variants might be beneficial is currently unknown, though some possibilities are apparent. Most psychiatric disorders and diseases can be viewed as a quantitative trait. In depression there is the extreme of low mood, pessimism and social withdrawal, though it is easy to imagine how an overly optimistic individual could also suffer reduced fitness from misjudging real risks and engaging in situations or individuals that were better avoided. Given the supposed association with stressful life events and loss of social support, depression may to some extent reflect an adaptive mechanism (or at least an adaptive mechanism malfunctioning) evolved to avoid risks when least able to deal with them. It is also worth noting that it has long been believed that psychiatric disorders, particularly psychosis, may overlap in families with beneficial traits. A large family studies of several hundred thousand in Sweden found that individuals with bipolar disorder and healthy siblings of people with schizophrenia and bipolar disorder are overrepresented in creative professions but those with major depression and their relatives are not [Kyaga and others 2011]. The results of this study provide support for the notion that creativity and bipolar disorder may co-segregate in families, however did not provide matching evidence for major depression.

Whether such associations with beneficial traits or gene-environments will provide novel insights into the genetic architecture of psychiatric disorders in the future is unclear. However the

increasing existence of large population datasets with access to detailed phenotypic, environmental and genomics data should allow future research to explore these questions and hopefully improve our understanding of the aetiologies of these disorders.

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